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- (71) Applicant (for all designated States except US): CONSORTIUM NATIONAL DE RECHERCHE EN GENOMIQUE (CNRG) [FR/FR]; 2, rue Gaston Crémieux, F-91000 Evry (FR).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): GUT, Ivo, Glynne [GB/FR]; 18, rue du Moulin Vert, F-75014 Paris (FR). KUCHARZAK, Ramon [FR/FR]; 56, rue Olivier Metra, F-75020 Paris (FR).
- (74) Agents: MARTIN, Jean-Jacques, et al.; Cabinet Regimbeau, 20, rue de Chazelles, F-75847 Paris Cedex 17 (FR).

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(54) Title: METHOD FOR HLA TYPING

(57) Abstract: A method for the identification of DNA sequence elements in complex and highly variable sequences is described. The method consists of identifying a short sequence element of several DNA bases (2-6 bases) at a given position in the genome simultaneously on all parental alleles. The method allows differentiating mini-haplotypes on different alleles in one analysis. The method consists of carrying out an enzymatic primer extension reaction with a combination of extension primers (pool of primers) and analysing the products by mass spectrometry. The pool of primers is assembled in such a way that the primer extension product allows unambiguous identification of both the primer of the pool that was extended and the base that was added. The method is of great utility for DNA sequences harbouring many SNPs close to each other with many possible haplotypes. Such sequences are known in the Major Histocompatibility Complex (MHC). This method is particularly well suited for DNA-based HLA typing and in combination with a suitable selection of sites tested, it is superior in ease of operation to conventional HLA typing methods. We have identified sets of these assays for HLA-A, HLA-B, and HLA-DRB 1 that allow unambiguous four-digit HLA of each of these genes with between 11 and 28 queried markers.

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#### Method for HLA typing

The present invention relates to a method for HLA typing by the unambiguous determination of short DNA sequence elements (2-6 bases) at a given position simultaneously on both parental alleles at a selected number of positions in HLA genes, comprised of the steps for each position of a) hybridising a combination of oligonucleotides (primers) complementary to all known sequence variants to a DNA strand upstream of a given position; b) carrying out a primer extension reaction with at least one of the four dNTP substrates substituted by a terminating analog; c) analysing the products by mass spectrometry, with the resulting masses allowing unambiguous identification of the used primers and the added bases. This method is particularly well suited for DNA-based HLA typing and in combination with a suitable selection of sites tested, it is superior in ease of operation to conventional HLA typing methods.

The most important of the genome projects, the complete sequence of the human genome, is finished. This project reveals the complete sequence of the 3 billion bases and the relative positions of all estimated 30.000 genes in this genome. Having this sequence opens unlimited possibilities for the elucidation of gene function and interaction of different genes. In recent years a systematic effort (SNP consortium) has been underway to identify single nucleotide polymorphisms (SNPs) throughout the human genome and so far several million of these differences between different human beings have been identified (dbSNP contained 5.5 million SNPs in October 2003).

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI) has revolutionized the mass spectrometric analysis of biomolecules (Karas, M. & Hillenkamp, F. Anal. Chem. 60, 2299-2301 (1988)). The field of DNA analysis by mass spectrometry was recently extensively reviewed by Tost and Gut (Mass Spectrometry Reviews, 21, 388-418 (2002)) and Sauer and Gut (Journal of Chromatography B, 782, 73-87, (2002)). MALDI has been applied to the analysis of DNA in variations that range from the analysis of PCR products to approaches using allele-specific termination to single nucleotide primer extension reactions and sequencing (Liu, Y.-H., et al. Rapid Commun. Mass Spectrom. 9, 735-743 (1995);

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Ch'ang, L.-Y., et al. Rapid Commun. Mass Spectrom. 9, 772-774 (1995); Little, D.P., et al. J. Mol. Med. 75, 745-750 (1997); Haff, L. & Smirnov, I.P. Genome Res. 7, 378-388 (1997), Fei, Z., Ono, T. & Smith, L.M. Nucleic Acids Res. 26, 2827-2828 (1998); Ross, P., Hall, L., Smirnov, I. & Haff, L. Nature Biotech. 16, 1347-1351 (1998); Ross, P.L., Lee, K. & Belgrader, P. Anal. Chem. 69, 4197-4202 (1997); Griffin, T.J., Tang, W. & Smith, L.M. Nature Biotech. 15, 1368-1372 (1997); Köster, H., Higgins, G.S & Little, D.P. US Patent 6,043,031). These methods are used to genotype previously identified mutations, SNPs, or insertion/deletions (indels). Spin column purification and/or magnetic bead technology, reversed-phase purification, or ion-exchange resins are frequently applied prior to mass spectrometric analysis.

The GOOD assay (IG Gut et S. Beck: US 6,268,812; IG Gut et al: US 6,503,710) is a method for SNP genotyping that uses MALDI mass spectrometry for detection (Sauer et al. 28, e13 and e100 (2000)). Allele-distinction is based on primer extension. In order to make products more amenable to MALDI analysis a substantial part of the primer is removed prior to mass spectrometric analysis. A further element that is included is charge tagging. This means that the final product is conditioned such that it carries either a single positive or a single negative charge. Generally this is achieved by alkylation of a phosphorothicate backbone and in some instances including a quaternary ammonium group to the penultimate base of the primer. The attachment of the quaternary ammonium group gives options for the design of multiplexes - individual SNPs can be moved up or down in the mass spectrum to achieve optimal resolution and separation.

The major histocompatibility complex (MHC) of humans is a cluster of genes on chromosome 6p21. It is of greatest importance as many diseases show association with genes in this region of the genome. All human leukocyte antigen (HLA) coding genes are found in the MHC. The HLA genes are highly variable and implicated in tissue transplantation, immunity and autoimmune disease such as diabetes, psoriasis, lupus, Crohn's disease, colitis, arthritis, and others. The HLA class I genes are HLA-A, HLA-B, HLA-C, ..... The HLA class II genes are HLA-DR, HLA-DP,....

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HLA typing methods differ dramatically in their approaches. Serological tests can be carried out but have only limited resolution. In the last 15 years the DNA sequence of the MHC has been extensively studied and high resolution typing now makes use of a wealth of DNA sequence information. Methods for DNA based HLA typing range from SSA (sequence specific amplification) where combinations of primers that are specific for different alleles are used to carry out PCR (US 5,545,526). Primers are combined in a way that the sizing of the PCR products allows unambiguous assignment of present base combinations. Multiple combinations are used to identify HLA types. The procedure works its way through a tree of combinations starting with a grouping into rough classes from where on further tests are carried out with specific reagents to subdivide in a class. This method is also known as SSP (sequence specific primers). An alternative method is termed SSOP (sequence specific oligonucleotide probes; US 6,503,707). Here a locus specific PCR is carried out followed by hybridisation with sequence specific oligonucleotide probes. As sequencing technology (and in particular the software for sequence calling) has dramatically improved over the last decade it now is also possible to gain a good degree of identification of HLA types by sequencing (WO 98/35059). Effectively a locus-specific PCR product is sequenced. Problems that arise here are that heterozygous individuals occasionally give rise to ambiguous haplotype calls that can not be resolved (Robinson, J.; Waller, M.J.; Marsh, St.G.E.: "Exon Identities and Ambiguous Typing Combinations"; IMGT/HLA Database; October 2003). The inclusion of allele-specific PCR helps achieve certainty. Resolution requires multiple products per locus to be generated and sequenced. However, as sequencing results can be very convoluted the interpretation in absence of allele-specific PCR can be cumbersome. All together the sequence-based typing requires many iterations in application. Reference strand mediated conformation analysis (RSCA) is a method used to study samples that potentially have a previously unknown sequence in their HLA (Correl et al., Tissue Antigens 56, 82-86, 2000). For a recent review for the reasoning of HLA typing as well as methodological advances see Petersdorf et al. (Tissue Antigens, 61, 1-11, 2003).

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The inventors have thus set themselves the task of providing an easy method for the simultaneous capture of all parental mini-haplotypes in highly polymorphic regions of genomes. The procedure has to be executable on a cost-effective genotyping platform. The method should be particularly applicable for HLA typing. It is an aim to resolve frequent and rare HLA alleles as well as possible.

The object of the present invention is a method for HLA typing by the unambiguous determination of short DNA sequence elements (2-6 bases) simultaneously on both parental alleles at a selected number of positions in HLA genes, comprised of the steps for each position of a) hybridising a combination of oligonucleotides (primer pool) complementary to all known sequence variants to a DNA strand upstream of a given position; b) carrying out a primer extension reaction with at least one of the four dNTP substrates substituted by a terminating analog; c) analysing the products by mass spectrometry, with the resulting masses allowing unambiguous identification of the used primers and the added bases.

#### In the present invention:

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- "HLA" means the human leukocyte antigen locus on chromosome 6p21, consisting of HLA genes (HLA-A, HLA-B, HLA-C, HLA-DRB1,...) that are used to determine the degree of matching, for example, between a recipient and a donor of a tissue graft.
- "HLA typing" means the identification of a known HLA allele of a given locus (HLA-A, HLA-B, HLA-C, HLA-DRB1,...).
- "HLA allele" means a nucleotide sequence within a locus on one of the two parental chromosomes.
  - "HLA-A" means the DNA sequence of exons 2 and 3 of the HLA-A gene.
  - "HLA-B" means the DNA sequence of exons 2 and 3 of the HLA-B gene.
  - "HLA-DRB1" means the DNA sequence of exon 2 of the HLA-DRB1 gene.
- "Polymorphism" means individual positions in a DNA sequence that exist in different variants.
  - "Haplotype" means the DNA sequence of one of the two alleles in a give region of the genome.

- "Mini-haplotype" means 2-6 contiguous bases on one parental allele.
- "Primer pools" or "pools of primers" means sets of primers that are used in one primer extension reaction. For each known HLA allele at least one primer is in the pool that is completely complementary in sequence. This assures perfect annealing. Mismatches that are more than 4 bases from the 3'end of the primer do not affect the results of the GOOD assay, as all of those bases are removed by 5'phosphodiesterase after the primer extension reaction. Primers of the pool containing mismatches in the last few bases are not extended by the DNA polymerase and thus not observable.
- "MALDI mass spectrometer" means a mass spectrometer that uses matrix-assisted laser desorption/ionization for the volatilisation of a sample and time-of-flight analysis for mass separation.
  - "Subgroup" means alleles, which are identical after the mini-haplotyping of the first set of selected positions. For the high resolution typing we resolve subgroups generated with 10 mini-haplotyping reactions. The criteria for resolving subgroups are: a) they still contain alleles with different two-digit types, b) subgroups with more than four alleles, and c) subgroups with frequent alleles (see list below).
- Here we show a methodology for the determination of sequence motifs of 2-6 bases 20 in very polymorphic regions of genomes. In principle this methods equates to the determination of mini-haplotypes of 2-6 bases. The individual parental minihaplotypes can be determined in one reaction without ambiguities. This methodology is applied to a chosen set of positions for HLA typing of HLA-A, HLA-B, and HLA-DRB1. The sets disclosed here have different purposes. First sets 25 of 19, 19, and 10 positions are suggested to distinguish a maximum of HLA alleles in HLA-A, HLA-B, and HLA-DRB1, respectively, with respect to differentiating alleles that are frequent in the general population from ones that are rare. The frequent alleles that were screened for are A\*0101, A\*0201, A\*0301, A\*2301, A\*2402, A\*2902, A\*3001 and A\*3002 for HLA-A, B\*0702, B\*0801, B\*1302, 30 B\*1501, B\*1801, B\*3501, B\*3503, B\*4001, B\*4402, B\*4403, B\*5101 and B.\*5701 for HLA-B, and DRB1\*0101, DRB1\*0301, DRB1\*0401, DRB1\*0701,

DRB1\*1101, DRB1\*1104, DRB1\*1302 and DRB1\*1501 for HLA-DRB1. This set of markers provides unambiguous identification of frequent HLA alleles with 93.4 - 100 % certainty in HLA-A, 97.6 - 100 % in HLA-B, and 97.2 - 100 % in HLA-DRB1.

A second set of 10 positions each in HLA-A, HLA-B, and HLA-DRB1, respectively are described that provide a maximum number of subgroups, that can then be further resolved by the addition of a set of subgroup specific positions. Again the ten positions in each locus were chosen on the basis of providing best distinction between the frequent HLA alleles listed above from the rest of the HLA alleles (rare). This resulted in groups containing 2-30 HLA alleles depending on the locus. Within each group a number of positions can be tested to provide resolution between the HLA alleles within the group. The number of positions that have to be additionally analysed range from 1-25 in order to achieve 4-digit resolution. With this technology HLA typing can be carried out at a substantially reduced cost with a proven high-throughput detection platform (MALDI mass spectrometry).

In a preferred embodiment of the method of the invention, the DNA strand of step a) is produced by a DNA replication procedure such as PCR or rolling circle replication.

A set of locus-specific PCR reactions for the selective amplification of each locus is described by the International Histocompatibility Working Group, Technical Manuals (www.ihwg.org/tmanual/Tmcontents.htm).

In a very preferred embodiment of the method of the invention, a combination of primers (pools of primers) contains slightly varying sequences so that all known sequences of the HLA alleles are accommodated by a perfectly matching primer.

The pool of primers guarantees that at least one primer is perfectly matched. The hybridised oligonucleotides of the primer pool are extended onto a polymorphic position. A requirement is that the added base together with the base composition of the primer gives a unique mass. The detection of this mass in the mass spectrometric profile indicates the presence of a sequence containing both the complementary sequence of the primer and the added base. In order to make all primers of a primer pool distinguishable by mass it is possible to add different mass

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shifting agents to the primers. The easiest way to accomplish this is by using charge/mass tagging technology such as is used in the GOOD assay. The penultimate base from the 3'end of the primer is amino-modified and used to add tags via NHS-ester chemistry. The pools of primers of course contain primers that sometimes differ by as little as one base. Sequences identical in base content can still be distinguished by the suitable selection of mass tags. Also, we have found that a primer carrying a mismatch in the last eight bases from the 3'end even if it anneals is not extended by the polymerase and thus screened out. This might be due to insufficient hybridisation or a resistance of the DNA polymerase to attach or extend when a mismatch is present. We thus make use of two effects for our minihaplotyping: 1) allele-specific hybridisation and 2) allele-specific primer extension. Mismatches that are further than four bases away from the 3'end of the extension primer do not result in increased complexity of the mass spectra as they are removed in the 5'phosphodiesterase digestion step of the GOOD assay.

In a preferred embodiment of the method of the invention, mass shifting tags are added to the individual primers sequences of a primer pool to make them uniquely distinguishable once the terminating base is added.

In another preferred embodiment of the method of the invention, termination products for know alleles are generated by extending the perfectly hybridised primer with a combination of dNTPs and ddNTPs or analogues thereof with a DNA polymerase to generate specific termination products to make them uniquely distinguishable by their mass.

In a preferred embodiment of the method of the invention, the GOOD assay is used. It typically applies single base primer extension, thus only the four terminating bases (ddNTPs) or synthetic analogues with the same qualities in terms of DNA polymerase tolerance are used for primer extension.  $\alpha$ -S-ddNTPs are very suitable analogues.

In a preferred embodiment of the method of the invention, mass spectrometry, in particular MALDI or ESI mass spectrometry is used for analysis of the masses of products.

For HLA typing a set of said mini-haplotyping assays has to be carried out to achieve sufficient information content.

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For HLA typing of HLA-A the preferred set of assays are those of positions 98, 414, 539, 282, 571, 368, 256, 292, 238, 270, 453, 527, 502, 81, 268, 559, 92, 123 and 396 (according to the numbering of the HLA-A gene starting at cDNA sequence position 1 of exon 1; see Figure 1). This results in medium resolution HLA typing. The input criteria for the selection are the frequency of HLA alleles.

Some HLA types are identified unambiguously.

For HLA typing of HLA-B accordingly the following positions are preferably analysed by mini-haplotyping assays to achieve medium resolution: 539, 419, 559, 412, 272, 362, 302, 363, 206, 369, 259, 97, 583, 292, 222, 527, 418, 435 and 571

(according to the numbering of the HLA-B gene starting at cDNA sequence 10 position 1 of exon 1; see Figure 2).

For HLA typing of HLA-DRB1 accordingly the following positions are preferably analysed by mini-haplotyping to achieve medium resolution: 125, 196, 197, 227, 261, 286, 299, 308, 341 and 345 (according to the numbering of the HLA-DRB1 gene starting at cDNA sequence position 1 of exon 1; see Figure 3).

In a preferred embodiment for high resolution HLA typing of HLA-A positions 98, 414, 539, 282, 571, 368, 256, 292, 238 and 270 (according to the numbering of the HLA-A gene starting at cDNA sequence position 1 of exon 1; see Figure 4) are used for mini-haplotyping to generate sub-groups (HLA-A\_A, HLA-A\_B, HLA-

- A\_C, HLA-A\_D, HLA-A\_E, HLA-A\_F, HLA-A\_G, HLA-A\_H, HLA-A\_I, HLA-20 A\_J, HLA-A\_K, HLA-A\_L, HLA-A\_M, HLA-A\_N, and HLA-A\_O; see Table I). Positions 224, 268, 376, 502, 561 and 616 are preferably analysed to resolve subgroup HLA-A\_A (sequences identical over exons 2 and 3 for alleles A\*29010101 and A\*29010102); positions 126 and 526 to resolve subgroup HLA-
- A\_B; positions 81, 90, 92, 212, 214, 257, 265, 299, 302, 404, 420, 427, 453, 485, 25 489 and 502 to resolve subgroup HLA-A\_C (sequences identical over exons 2 and 3 for alleles A\*24020101, A\*24020102L, A\*240203, A\*2409N and A\*2411N); positions 160, 200, 362 and 524 to resolve subgroup HLA-A\_D; positions 180, 299, 301, 302, 346, 418, 453, 517, 524, 526, 527, 557, 559 and 560 to resolve subgroup
- HLA-A\_E; positions 299, 301, 302, 341 and 583 to resolve subgroup HLA-A F; 30 positions 127, 341, 399, 480, 502, 503, 524, 526, 527, 553, 559, 560 and 565 to resolve subgroup HLA-A\_G; positions 228, 233, 463, 519, 530 and 583 to resolve

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subgroup HLA-A\_H; positions 102, 275, 317, 362, 418, 419, 497, 524, 555, 595 and 618 to resolve subgroup HLA-A\_I (sequences identical over exons 2 and 3 for alleles A\*680102 and A\*6811N); positions 92, 331, 453, 524, 559, 560 and 564 to resolve subgroup HLA-A\_I; positions 78, 81, 123, 125, 142, 144, 194, 268, 294, 324, 355, 362, 396, 403, 419, 453, 456, 477, 493, 517, 524, 526, 527, 559 and 560 to resolve subgroup HLA-A\_K (sequences identical over exons 2 and 3 for alleles A\*02010101, A\*02010102, A\*020108, A\*0209, A\*0243N and A\*0266); positions 113, 299, 301, 302, 308, 311, 523, 524 to resolve subgroup HLA-A\_L; positions 171, 363, 498 and 559 to resolve subgroup HLA-A\_M; positions 376, 426, 527, 555, 557 and 595 to resolve subgroup HLA-A\_N; position 299 to resolve subgroup HLA-A\_O.

<u>TABLE I</u>

Subgroups of	Alleles of Subgroups	Positions to resolve
HLA-A	·	Subgroups
HLA-A_A	A*29010101, A*29010102, A*290201, A*290202, .	224, 268, 376, 502, 561,
	A*2904, A*2906, A*2908N, A*2909	616
HLA-A_B	A*3002, A*3009, A*3012	126, 526
HLA-A_C	A*24020101, A*24020102L, A*240202, A*240203, A*240204, A*2404, A*2405, A*2408, A*2409N,	81, 90, 92, 212, 214, 257, 265, 299, 302, 404, 420,
	A*2411N, A*2420, A*2421, A*2425, A*2426, A*2427,	427, 453, 485, 485, 489, 502
	A*2429, A*2432, A*2435, A*2436N, A*2437, A*2438,	
	A*2439	
HLA-A_D	A*0206, A*0214, A*0221, A*0251, A*0257	160, 200, 362, 524
HLA-A_E	A*250101, A*250102, A*2601, A*2604, A*2605,	180, 299, 301, 302, 346,
	A*2609, A*2610, A*2611N, A*2612, A*2614, A*2615,	418, 453, 517, 524, 526, 527, 557, 559, 560
}	A*2617, A*2618, A*6603	
HLA-A_F	A*2502, A*2613, A*6601, A*6602, A*6604	299, 301, 302, 341, 583
HLA-A_G	A*110101, A*110102, A*1102, A*1103, A*1104,	127, 341, 399, 480, 502,
	A*1105, A*1107, A*1109, A*1112, A*1113, A*1114,	503, 524, 526, 527, 553, 559, 560, 565
	A*1115	
HLA-A_H	A*3301, A*330301, A*330302, A*3304, A*3305,	228, 233, 463, 519, 530, 583
	A*3306, A*3307	
HLA-A_I	A*680101, A*680102, A*680103, A*6807, A*6811N,	102, 275, 317, 362, 418, 419, 497, 524, 555, 595,
	A*6812, A*6816, A*6817, A*6819, A*6821, A*6822,	618
	A*6823, A*6824	00 221 452 524 556
HLA-A_J	A*2301, A*2303, A*2305, A*2306, A*2307N,	92, 331, 453, 524, 556, 560, 564
	A*2308N, A*2310, A*2413	
HLA-A_K	A*02010101, A*02010102, A*020102, A*020103,	78, 81, 123, 125, 142, 144, 194, 268, 294, 324,
	A*020104, A*020105, A*020106, A*020107, A*020108, A*020109, A*0204, A*0209, A*0216,	355, 362, 396, 403, 419, 453, 419, 453, 456, 477,
	A*0224, A*0225, A*0226, A*0229, A*0230, A*0231,	493, 517, 524, 526, 527,
	A*0232N, 0A*0240, A*0242, A*0243N, A*0258,	559, 560
	A*0259, A*0260, A*0264, A*0266, A*0267, A*0253N	
HLA-A_L	A*3201, A*3203, A*3206, A*7401, A*7402, A*7403,	113, 299, 301, 302, 308,
	A*7408, A*7409	311, 523, 524
HLA-A_M	A*010101, A*010102, A*0103, A*0104N, A*0108,	171, 363, 498, 559
_	A*0109	
HLA-A_N	A*03010101, A*03010102, A*0303N, A*0304, A*0305,	376, 426, 527, 555, 557,
	A*0306, A*0307, A*0311N	595
HLA-A_O	A*2504, A*2608	299

In a preferred embodiment for high resolution, HLA typing of HLA-B positions 539, 419, 559, 412, 272, 362, 302, 363, 206 and 369 (according to the numbering of the HLA-B gene starting at cDNA sequence position 1 of exon 1; see Figure 5) are used for mini-haplotyping to generate sub-groups (HLA-B A, HLA-B B, HLA-B C, HLA-B D, HLA-B\_E, HLA-B\_F, HLA-B\_G, HLA-B\_H, HLA-B\_I, HLA-B\_J, 5 HLA-B\_K, HLA-B\_L, HLA-B\_M, HLA-B\_N, HLA-B\_O, HLA-B\_P, HLA-B\_Q, HLA-B R, HLA-B\_S, HLA-B\_T, HLA-B\_U, HLA-B\_V, HLA-B\_W, HLA-B\_X, -HLA-B\_Y, HLA-B\_Z, HLA-B\_AA, HLA-B\_AB and HLA-B\_AC; see Table II). Positions 259, 341 and 473 are preferably analyzed to resolve subgroup HLA-B\_A (sequences identical over exons 2 and 3 for alleles B\*0801 and B\*0819N); positions 10 106, 144, 222, 259, 273, 311, 313, 418, 445, 493, 528 and 540 to resolve subgroup HLA-B B (sequences identical over exons 2 and 3 for alleles B\*44020101, B\*44020102, B\*4419N and B\*4427); positions 319, 416, 545 and 572 to resolve subgroup HLA-B C; positions 106, 131, 165, 215, 243, 277, 292, 322, 481, 582, 603 and 616 to resolve subgroup HLA-B D; positions 106, 146, 165, 181, 238, 259, 263, 15 292, 328.1/329(insert for B\*1579N), 379, 435, 453, 463, 485, 526, 571, 572 and 583 to resolve subgroup HLA-B E (sequences identical over exons 2 and 3 for alleles B\*15010101 and B\*15010102); positions 142, 171, 255, 257, 395, 430, 544, 566 and 572 to resolve subgroup HLA-B\_F; positions 117, 247, 248, 277, 345, 418, 489 and 527 to resolve subgroup HLA-B G (sequences identical over exons 2 and 3 for alleles 20 B\*270502, B\*270504 and B\*2713); positions 134, 141, 200, 213, 259, 304 and 527 to resolve subgroup HLA-B\_H; positions 83, 141, 211, 222, 242, 322, 404, 414, 435, 463, 502, 527, 544, 571, 572 and 583 to resolve subgroup HLA-B\_I (sequences identical over exons 2 and for alleles B\*510101, B\*510105, B\*5111N, B\*5130 and B\*5132); positions 103, 142, 222, 243, 259, 292, 477, 486 and 499 to resolve 25 subgroup HLA-B\_J (sequences identical over exons 2 and 3 for alleles B\*400101 and B\*400102); positions 103, 259, 292, 295, 527 and 583 to resolve subgroup HLA-B\_K (sequences identical over exons 2 and 3 for alleles B\*180101 and B\*1817N); positions 320 and 500 to resolve subgroup HLA-B\_L; positions 311, 527 and 583 to resolve subgroup HLA-B\_M; positions 119, 292, 259, 319, 425, 527, 546 and 583 to 30 resolve subgroup HLA-B N (sequences identical over exons 2 and 3 for alleles B\*350101, B\*3540N and B\*3542); positions 97, 142, 245 and 527 to resolve subgroup HLA-B O; positions 97 and 175 to resolve subgroup HLA-B\_P; positions

## TABLE II

Subgroups of	Alleles of the subgroup	Positions to resolve
HLA-B		<u>Subgroups</u>
HLA-B_A	B*0801, B*0808N, B*0810, B*0818, B*0819N	259, 341, 473
HLA-B_B	B*44020101, B*44020102S, B*440202, B*440203,	106, 144, 222, 259, 273
	B*4405, B*4411, B*4412, B*4419N, B*4422, B*4423N,	311, 313, 418 445, 493,
	B*4424, B <b>*</b> 4425, B <b>*</b> 4427, B <b>*</b> 4433, B <b>*</b> 4434, B <b>*</b> 4435	528, 540
HLA-B_C	B*4415, B*4501, B*4503, B*4504, B*4505	319, 416, 545, 572
HLA-B_D	B*070201, B*070202, B*070203, B*070204, B*0703,	106, 131, 165, 215, 243,
	B*0716, B*0721, B*0722, B*0723, B*0729, B*0730,	277, 292, 322, 481, 582,
	B*0733, B*0735	603,616
HLA-B_E	B*15010101, B*15010102, B*150102, B*150103,	106, 146, 165, 181, 238,
	B*150104, B*1512, B*1514, B*1515, B*1519, B*1528,	259, 263, 292,
	B*1533, B*1534, B*1538, B*1560, B*1570, B*1571,	328.1/329, 379, 435,
	B*1575, B*1578, B*1579N, B*1581, B*1582	453, 463, 485, 526, 571,
	• .	572,583
HLA-B_F	B*440301, B*4413, B*4426, B*4429, B*4430, B*4432,	142, 171, 255, 257, 395,
	B*4436, B*4437, B*4438, B*4439	430, 544, 566 , 572
HLA-B_G	B*2703, B*270502, B*270503, B*270504, B*270505,	117, 247, 248, 277, 345,
	B*270506, B*2709, B*2710, B*2713, B*2716, B*2717	418, 489 , 527
HLA-B_H	B*5107, B*520101, B*520102, B*520103, B*520104,	134, 141, 200, 213, 259,
	B*5203, B*5204, B*5205	304,527
HLA-B_I	B*510101, B*510102, B*510103, B*510104, B*510105,	83, 141, 211, 222, 242,
	B*510201, B*510202, B*5103, B*5109, B*5111N,	322, 404, 414, 435, 463,
	B*5112, B*5114, B*5118, B*5119, B*5123, B*5124,	502, 527, 544, 571, 572 ,
	B*5126, B*5127N, B*5128, B*5130, B*5132, B*5133	583
HLA-B_J	B*400101, B*400102, B*400103, B*4010, B*4011,	103, 142, 222, 243, 259,
	B*401401, B*401402, B*401403, B*4022N, B*4025,	292, 477, 486 , 499
	B*4043	
HLA-B_K	B*180101, B*180102, B*1803, B*1804, B*1805,	103, 259, 292, 295, 527,
	B*1811, B*1812, B*1815, B*1817N	583
HLA-B_L	B*570101, B*5706, B*5708	320, 500
HLA-B_M	B*3527, B*5301, B*5302, B*5306, B*5308	311, 527 , 583
HLA-B_N	B*350101, B*350102, B*3507, B*3510, B*3511,	119, 292, 259, 319, 425,
	B*3521, B*3524, B*3529, B*3540N, B*3541, B*3542,	527, 546, 583
	B*5305	
HLA-B_O	B*5501, B*5502, B*5505, B*5510, B*5516	97, 142, 245, 527
HLA-B_P	B*5401, B*5402, B*5507	97,175

HLA-B_Q	B*3910, B*670101, B*670102	246, 277
HLA-B_R	B*3803, B*390201, B*390202, B*3913, B*3923	246, 292, 311, 503
HLA-B_S	B*3801, B*380201, B*380202, B*3804, B*3805, B*3809	103, 261, 309, 311, 474
HLA-B_T	B*390101, B*390103, B*390104, B*3904, B*3905,	97, 103, 106, 243, 259,
	B*3912, B*3922, B*3925N, B*3926	292, 404 , 524
HLA-B_U	B*3503, B*3513, B*3536	259,320
HLA-B_V	B*0734, B*5504	106
HLA-B_W	B*4047, B* 4431	97
HLA-B_X	B*4002, B*4027, B*4029, B*4035, B*4040, B*4045	97, 106, 257, 418, 463
HLA-B_Y	B*400104, B*4004	106
HLA-B_Z	B*4012, B*4046, B*4803	106, 144
HLA-B_AA	B*2703, B*270502, B*270503, B*270504, B*270505,	117, 247, 248, 283, 345,
	B*270506, B*2709, B*2710, B*2713, B*2716, B*2717	418, 489, 527
HLA-B_AB	B*1562, B*4802	106
HLA-B_AC	B*1302, B*1308	548

246 and 277 to resolve subgroup HLA-B\_Q; positions 246, 292, 311 and 503 to resolve subgroup HLA-B\_R; positions 103, 261, 309, 311 and 474 to resolve subgroup HLA-B\_S; positions 97, 103, 106, 243, 259, 292, 404 and 524 to resolve subgroup HLA-B\_T (sequences identical over exons 2 and 3 for alleles B\*390101 and B\*390103); positions 259 and 320 to resolve subgroup HLA-B\_U; position 106 to resolve HLA-B\_V; positions 97 to resolve HLA-B\_W; positions 97, 106, 257, 418 and 463 to resolve HLA-B\_X; position 106 to resolve HLA-B\_Y; positions 106 and 144 to resolve HLA-B\_Z; positions 117, 247, 248, 283, 345, 418, 489, and 527 to resolve HLA-B\_AA; positions 106 to resolve HLA-B\_AB; positions 548 to resolve HLA-B\_AA.

In a preferred embodiment, the method for HLA typing resolves groups A-P of HLA-DRB1.

For high resolution, HLA typing of HLA-DRB1 positions are: 125, 196, 197, 227, 261, 286, 299, 308, 341 and 345 (according to the numbering of the HLA-DRB1 gene starting at DNA sequence position 1 of exon 1; see Figure 6) are used for minihaplotyping to generate sub-groups (HLA-DRB1\_A, HLA-DRB1\_B, HLA-DRB1\_C, HLA-DRB1\_D, HLA-DRB1\_E, HLA-DRB1\_F, HLA-DRB1\_G, HLA-DRB1\_H, HLA-DRB1\_I, HLA-DRB1\_J, HLA-DRB1\_K, HLA-DRB1\_L, HLA-DRB1\_M, HLA-DRB1\_I, HLA-DRB1\_O, HLA-DRB1\_P; see Table III).

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In a very preferred embodiment, positions 123, 174, 250, 278 and 317 are analysed to resolve subgroup HLA-DRB1 A; positions 192, 203, 256 and 259 to resolve subgroup HLA-DRB1 B; 256, 260, 317 and 351 to resolve subgroup HLA-DRB1 C; positions 155, 204, 233, 239, 256, 304, 357 and 366 to resolve subgroup HLA-DRB1 D; positions 122, 171, 257 and 317 to resolve subgroup HLA-DRB1 E; positions 164, 167, 171, 230, 235, 306, 317, 321 and 337 to resolve subgroup HLA-DRB1 F; positions 164, 257, 266 and 303 to resolve subgroup HLA-DRB1 G; positions 164, 181, 188, 220, 229, 256, 266, 317 and 318 to resolve subgroup HLA-DRB1 H; position 257 to resolve subgroup HLA-DRB1 I; positions 181, 239 and 357 to resolve subgroup HLA-DRB1 J; positions 122, 144, 239, 303, 317, 318 and 321 to resolve subgroup HLA-DRB1 K (sequences identical over exons 2 and 3 for alleles DRB1\*110101 and DRB1\*110102); positions 118, 161, 257, 260, 318 and 321 to resolve subgroup HLA-DRB1 L; positions 165, 257, 293 and 303 to resolve subgroup HLA-DRB1 M (sequences identical over exons 2 and 3 for alleles DRB1\*120101 and DRB1\*1206); positions 177, 240, 256, 257 and 357 to resolve subgroup HLA-DRB1 N; positions 150 175, 230, 236 and 321 to resolve subgroup HLA-DRB1 O (sequences identical over exons 2 and 3 for alleles DRB1\*150101 and DRB1\*1513); positions 115, 220 and 317 to resolve subgroup HLA-DRB1\_P.

Another object of the invention is a kit to carry out the procedure. It consists of pooled combinations of primers. The primers that are used in the pools for HLA-A, HLA-B, and HLA-DRB1 and the masses of the genotyping products are listed in Tables IV, V, and VI respectively. CT refers to the mass shifting mass tag that is attached to that primer of the pool.

Another object of the invention is the use of the method of the invention for screening of tissue donors.

In a preferred embodiment, the use is for bone marrow donors in registries for screening of frequent and rare HLA types.

Still another object of the invention is the use of the primers represented in Table IV, V and VI to carry out HLA typing.

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### TABLE III

Subgroups of	Alleles of Subgroups	Positions to resolve			
HLA-DRB1		Subgroups			
HLA-	DRB1*070101, DRB1*070102, DRB1*0703, DRB1*0704,	14, 123, 174, 250, 317			
DRB1_A	DRB1*0705, DRB1*0707				
HLA-	DRB1*040101, DRB1*040102, DRB1*0409, DRB1*0426,	192, 203, 256, 259			
DRB1_B	DRB1*0433				
HLA-	DRB1*0404, DRB1*0410, DRB1*0423, DRB1*0440,	256, 260, 317, 351.			
DRB1_C	DRB1*0444				
HLA-	DRB1*040501, DRB1*040502, DRB1*040503,	155, 204, 233, 239,			
DRB1_D	DRB1*040504, DRB1*0408, DRB1*0429, DRB1*0430,	256, 304, 357, 366			
	DRB1*0445, DRB1*0448				
HLA-	DRB1*1402, DRB1*1409, DRB1*1413, DRB1*1446,	122, 171, 257, 317			
DRB1_E	DRB1*1447, DRB1*1448				
HLA-	DRB1*130101, DRB1*130102, DRB1*130103,	164, 167, 171, 230,			
DRB1_F	DRB1*1315, DRB1* 1327,	235, 306, 317, 321,			
1		337			
HLA-	DRB1*130201, DRB1*130202, DRB1*1331, DRB1*1339,	164, 257, 266, 303			
DRB1_G	DRB1*1341	·			
HLA-	DRB1*030101, DRB1*030102, DRB1*0307, DRB1*0312,	164, 181, 188, 220,			
DRB1_H	DRB1*0313, DRB1*0315, DRB1*0316, DRB1*0318,	229, 256, 266, 317,			
	DRB1*0322, DRB1*0323	318			
HLA-	DRB1*1137, DRB1*1425	257			
DRB1_I					
HLA-	DRB1*110401, DRB1*110402, DRB1*1143, DRB1*1146	181, 239, 357			
DRB1_J					
HLA-	DRB1*110101, DRB1*110102, DRB1*110103,	122, 144, 239, 303,			
DRB1_K	DRB1*110104, DRB1*110105, DRB1*112701,	317, 318, 321			
	DRB1*112702, DRB1*1130, DRB1*1139				
HLA-	DRB1*1117, DRB1*140101, DRB1*140102, DRB1*1408,	118, 161, 257, 260,			
DRB1_L	DRB1*1426, DRB1*1438, DRB1*1439	318, 321			
HLA-	DRB1*120101, DRB1*120102, DRB1*1206, DRB1*1207,	165, 257, 293, 303			
DRB1_M	DRB1*1208, DRB1*1209				
HLA-	DRB1*080101, DRB1*080102, DRB1*080201,	177, 240, 256, 257,			
DRB1_N	DRB1*080202, DRB1*080203, DRB1*0807, DRB1*0811	357			
HLA-	DRB1*150101, DRB1*150103, DRB1*150105,	, 150 175, 230, 236,			
DRB1_O	DRB1*1503, DRB1*1506, DRB1*1509, DRB1*1513	321			
HLA-	DRB1*010101, DRB1*0105, DRB1*0107, DRB1*0111	115, 220, 317			
DRB1_P	$\mathcal{P}_{i}$				

TABLE IV

No.   Name   Sequence   CT   Masses			TABLE IV		Primer	A	С	G	T
National				СТ		A	C	G	1
1   1.1.4.0   11.00						1425 4	4404 21	——-т	
The content of the									
Second Color	2	HLAA_812_1f20	TGCTCGCCCCAGGCTC1spC^spA	10	1113,1		1410,3	1432,4	
HILAA 922 1120   AGGCTCCAMTCCATGAGSpG-9pT   0   1199,1   1496,4   1512,4     5   HILAA 923 1120   AGGCTCTCASTCCATGAGSpG-9pT   0   1169,1   1496,4   1512,4     6   HILAA 923 1120   CCACTCCATGAGGTATTTSpC-8pA   0   1113,1   1496,4   1512,4     7   HILAA 923 1120   CCACTCCATGAGGTATTTSpC-8pA   0   1113,1   1496,4   1407,3   -				-	4420 4	AAEC A			
1.   1.   1.   1.   1.   1.   1.   1.		1						15124	<del></del> -
S			AGGCTCCCAMTCCATGAGspG^sp1						
0         THLAA         982_1120         COACTCCATGAGGTATTTSpC*spT         0         1104,1         1431,4         1407,3           8         HLAA         1231_2120         GCGATGAGCGGGGCTCspCspT*spC*spC         0         1510,5         -         -         1853,8           9         HLAA         1232_220         GGGATGAGCGGGGCTCspTspC*pC*spC         0         1468,4         -         1751,6           10         HLAA         1233_220         GGGATGAGCGGGGCTCspCspC*pSpC         0         1497,4         1800,6         -         1751,6           11         HLAA         2331_220         CTGGTCCAATACTCCGspGspA*spC         0         1497,4         1800,6         -           13         HLAA         2381_220         CTGGTCCAATACTCCGspGspC*spC         0         1497,4         1800,6         -           14         HLAA         2383_220         CTGGTCCAATACTCCGspGspC*spC         0         1473,4         1776,6         -           15         HLAA         2383_220         CTGGTCCAATACTCCGspGspC*spC         0         1473,4         1776,6         -           17         HLAA         2385_220         CWGGTCCAATACTCCGspGspC*spC         0         1473,4         1776,6         -           18	5	HLAA_923_1f20	AGGCTCTCASTCCATGAGspG^sp1	1-	1105,1	1450,4		1312,4	
THLAA 982   1120   CCACTCCATGAGGTATTTSpC*spT   0   1104,1   1431,4   1407,3				1	44424		1415 2		
R   HLAA   1231   1270   GCGATGAAGCGGGGCTCspCspT*spC   0   1510.5   -   1853.8     R   HLAA   1232   1270   GCGATGAAGCGGGGCTCspT*spC*spC   28   1380.4   1707.7   -   1751.6     R   HLAA   1232   1270   GCGATGAAGCGGGGCTTspCspC*spC   0   1408.4   -   1751.6     R   HLAA   1234   1270   GMGATGAAGCGGGGCTTspCspC*spC   0   1408.4   -   1751.6     R   HLAA   1234   1270   GMGATGAAGCGGGGCTTspCspC*spC   0   1393.4   1720.7   1736.7     R   HLAA   1234   1270   GMGATGAAGCGGGGCTTspCspC*spC   0   1497.4   -   1800.6   -     1800.6   -     1800.6   -     1800.6   -     1800.6   -						4424 4		<del></del>	1422,3
8 HILAA 1232 2720 GCGATGAAGCGGGGCTCspTspC*spC 28 1380.4 1707.7 - 1751.6 10 HILAA 1233 2720 GCGATGAAGCGGGGCTTspCspC*spC 0 1409.4 - 1751.6 11 HILAA 1233 2720 GMGATGAAGCGGGGGCTTspCspC*spC 0 1393.4 1720.7 - 1736.7 1	7	HLAA_982_1f20	CCACTCCATGAGGTATTTspC^sp1	10	1704,1	1431,4	1407,3	<del></del>	1422,5
8 HILAA 1232 1270 GCGATGAAGCGGGGCTCspTspC*spC 28 1380.4 1707.7 - 1751.6 10 HILAA 1232 1270 GCGATGAAGCGGGGCTTspCspC*spC 0 1408.4 - 1751.6 11 HILAA 1233 1270 GMGATGAAGCGGGGCTTspCspC*spC 0 1393.4 1720.7 - 1736.7 11 HILAA 1234 1270 GMGATGAAGCGGGGCTCspCspC*spC 0 1393.4 1720.7 - 1736.7 12 HILAA 2381 2720 GYGGTCCAATACTCCGSpGspA*spC 0 1497.4 - 1800.6 - 13 HILAA 2382 2720 GYGGTCCAATACTCCGSpGspA*spC 0 1497.4 - 1800.6 - 1497.4 - 1800.6 - 15 HILAA 2383 1270 GTGCTCCAATACTCCGSpGspC*spT 0 1488.4 - 1791.6 - 16 HILAA 2383 1270 GTGCTCCAATACTCCGSpGspC*spT 0 1488.4 - 1791.6 - 16 HILAA 2384 1270 CTSGTCCCAATACTCCGSpGspC*spC 0 1473.4 - 1776.6 - 16 HILAA 2385 1270 CYGGTCCAATACTCCGSpGspC*spC 0 1473.4 - 1776.6 - 17 HILAA 2385 1270 CYGGTCCAATACTCCGSpGspC*spC 0 1473.4 - 1776.6 - 17 HILAA 2385 1270 CYGGTCCAATACTCCGSpGspC*spC 0 1473.4 - 1776.6 - 18 HILAA 2385 1270 CYGGTCCAATACTCCGSpGspC*spC 0 1473.4 - 1776.6 - 1776.6 - 18 HILAA 2385 170 CMGGTCCCAATACTCCGSpGspC*spC 0 1473.4 - 1776.6 - 18 HILAA 2585 119 CTTCACATACTCCGTGTCTCSpC*spT 0 1089.1 - 1392.3 1432.4 20 HILAA 2565 119 CTTCACATKCCGTGTCTCSpC*spT 0 1089.1 - 1392.3 1432.4 120 HILAA 2565 119 CTTCACATKCCGTGTCTTSpC*spC 0 1089.1 - 1392.3 1432.4 120 HILAA 2565 119 CTTCACATKCCGTGTCTTSpC*spC 0 1089.1 - 1432.1 120 HILAA 2565 119 CTTCACATKCCGTGTCTTSpC*spC 0 1089.1 - 1432.1 120 HILAA 2565 119 CTTCACATKCCGTGTCTTSpC*spC 0 1089.1 - 1432.1 120 HILAA 2565 119 CTTCACATKCCGTGTCTTSpC*spC 0 1089.1 - 1432.1 120 HILAA 2565 119 CTTCACATKCCGTGTCTTSpC*spC 0 1089.1 - 1432.1 120 HILAA 2565 119 CTTCACATKCCGTGTCTCSpC*spC 0 1074.1 - 1377.3 1417.4 126 HILAA 2565 119 CTTCACATKCCGTGTCTCSpC*spC 0 1074.1 - 1377.3 1417.4 126 HILAA 2565 119 CTTCACATKCCGTGTCTCSpC*spC 0 1074.1 - 1377.3 1417.4 126 HILAA 2565 119 CTTCACATTCCGTGTCTTSpC*spC 0 1089.1 - 1432.1 120 HILAA 2565 119 CTTCACTTCCGTGTCTTSpC*spC 0 1098.1 - 1432.1 120 HILAA 2565 119 CTTCACTTCCGTGTCTTSpC*spC 0 1098.1 - 1432.1 120 HILAA 2565 119 CTTCACATTCCGTGTCTTSpC*spC 0 1098.1 - 1441.4 1457.3 - 1441.3 1441.3 1441.3 1441.3 1441.4 1441.4 1441.4 1441.4 1441.4 1441.4 1441.4 1441.				1-	4540 E			1052 0	
10   HLAA   1233   2720   GCGATGAAGCGGGGCTTSPCSPC^SPC   0   1408.4   -   1756.6   1756.7     11   HLAA   1234   2720   GTGGTCCAATACTCCGSPGSPA^SPC   0   1393,4   1720,7   1736,7     12   HLAA   2381   2720   CTGGTCCCAATACTCCGSPGSPA^SPC   0   1497,4   1800,6   -     13   HLAA   2382   2720   CYCGTCCCAATACTCCGSPGSPA^SPC   0   1497,4   1800,6   -	8	HLAA_1231_2r20	GCGATGAAGCGGGGCTCspCspT^spC			4707 7		1000,0	<del></del>
III   HLAA   1234   2720   GMGATGAAGGGGGGCTCSpCspC*SpC   0   1393,4   1720,7   1736,7	9	HLAA_1232_2r20	GCGATGAAGCGGGGCTCspTspC^spC	1				1751 6	
HLAA 2381 2720   CTSGTCCCAATACTCCGSpGspA*spC   0   1497,4   - 1800,6   - 13   HLAA 2382 2720   CYGGTCCCAATACTCCGSpGspA*spC   0   1497,4   - 1800,6   - 14   HLAA 2382 2720   CTGGTCCCAATACTCCGSpGspC*spT   0   1488,4   - 1791,6   - 15   HLAA 2385 2720   CTGGTCCCAATACTCCGSpGspC*spC   0   1473,4   - 1776,6   - 176   HLAA 2385 2720   CYGGTCCCAATACTCCGSpGspC*spC   0   1473,4   - 1776,6   - 176   HLAA 2385 2720   CYGGTCCCAATACTCCGSpGspC*spC   0   1473,4   - 1776,6   - 176   HLAA 2385 2720   CYGGTCCCAATACTCCGSpGspC*spC   0   1473,4   - 1776,6   - 176   HLAA 2387 2720   CYGGTCCCAATACTCCGSpGspC*spC   0   1473,4   - 1776,6   - 1776,6   - 176   HLAA 2387 2720   CYGGTCCCAATACTCGSpGspC*spC   0   1473,4   - 1776,6   - 1	10	HLAA_1233_2r20	GCGATGAAGCGGGGCTTspCspC^spC	4					<del></del> -
IHLAA 2382   120   CYCGTCCCAATACTCCGSPGSPA*SPC   0   1497.4   1800.6   18   14   14   14   14   14   14   14	11	HLAA_1234_2r20	GMGATGAAGCGGGGCTCspCspC^spC	U	1393,4	1720,7		1730,1	
					4407.4		4000 6		
13   HLAA 2383 2720   CTCGTCCCAATACTCCGSpGspC^spC	12	HLAA_2381_2r20	CTSGTCCCAATACTCCGspGspA^spC					<del></del>	<u></u>
15   HLAA 2384 2720   CTSGTCCCAATACTCAGSPGSPC^spC   0   1473,4   -   1776,6   -     16   HLAA 2385 2720   CYGGTCCCAATACTCCGSPGSPC^spC   0   1473,4   -   1776,6   -     17   HLAA 2385 2720   CMGGTCCCAATACTCCGSPGSPC^spC   0   1473,4   -   1776,6   -     18   HLAA 2387 2720   CMGGTCCCAATACTCCGSPGSPC^spC   0   1473,4   -   1776,6   -     19   HLAA 2387 2720   CYCGTCCCAATACTCCGSPGSPC^SpC   0   1473,4   -     1776,6   -     19   HLAA 2561 1119   CTTCACMTTCCGTGTCTCSPC^spT   0   1089,1   -   1392,3   1432,4     20   HLAA 2562 1119   CTTCACMTTCCGTGTCTCSPC^spT   0   1089,1   -   1392,3   1432,4     21   HLAA 2563 1119   CTTCACMTTCCGTGTCTSPC^spT   0   1089,1   -   1392,3   1432,4     22   HLAA 2564 1119   CTTCACMTTCCGTGTGTSPC^spC   0   1089,1   -   1432,1     23   HLAA 2565 1119   CTTCACATTCCGTGTGTTSPC^spC   0   1089,1   -   1432,1     24   HLAA 2566 1119   CTTCACATTCCGTGTGTTSPC^spC   0   1074,1   -   1377,3   1417,4     25   HLAA 2565 1119   CTTCACATTCCGTGTGTTSPC^spC   0   1074,1   -   1377,3   1417,4     26   HLAA 2565 1119   CTTCACATTCCGTGTCTSPC^spC   0   1074,1   -   1377,3   1417,4     27   HLAA 2568 1119   CTTCAGTTCCSPCTCTCSPC^spC   0   1074,1   -   1377,3   1417,4     28   HLAA 2568 1119   CTTCAGTTCCSPCTSPC   0   1074,1   -   1377,3   1417,4     28   HLAA 2568 1110   ATTGGGACCGGAACACACSPC^spC   0   1154,1   1481,4   1457,3   -     29   HLAA 2683 1120   ATTGGGACCTGCAGACACSPC^spC   0   1154,1   1481,4   1457,3   -     30   HLAA 2683 1120   ATTGGGACSAGGAGACACSPC^spG   0   1154,1   1481,4   1457,3   -     31   HLAA 2701 1119   CTGTGAGTGGGCCTTCSPA^spC   0   1194,1   1491,4   1457,3   -     32   HLAA 2824 1120   ATTGGGACSAGGAGACACSPC^spG   0   1194,1   1491,4   1457,3   -     33   HLAA 2701 1119   CTGTGAGTGGGCCTTCSPA^spC   -14   1084,1   1411,4   -   1427,4   -       34   HLAA 2702 1119   CTGTGAGTGGGCCTTCSPA^spC   -14   1084,1   1411,4   -   1427,4   -       35   HLAA 2824 1120   ACACGGAATGTGAGGCCSPC^spA   0   1098,1   -   1401,3   1441,3         36   HLAA 2821 1720   ACACGGAATGTGAGGCCSPC^spA   0   1098,1   -   1401,3	13	HLAA_2382_2r20	CYCGTCCCAATACTCCGspGspA^spC						1806,4
In   In   In   In   In   In   In   In	14	HLAA_2383_2r20	CTCGTCCCAATACTCCGspGspC^sp1						1000,4
17   HLAA   2385   2720   CMGGTCCCAATACTCCGspGspC^spC   0   1473,4   -   1776,6   -   18   HLAA   2387   2720   CYCGTCCCAATACTCCGspGspC^spC   0   1473,4   -   1776,6   -     18   HLAA   2387   2720   CYCGTCCCAATACTCCGSpGspC^spC   0   1473,4   -   1776,6   -     18   HLAA   2581   1719   CTTCACATTCCGTGTCTCspC^spT   0   1089,1   -   1392,3   1432,4   20   HLAA   2561   1719   CTTCACATTCCGTGTCTCspC^spT   0   1089,1   -   1392,3   1432,4   114,4   2563   1719   CTTCACATTCCGTGTCTCspC^spC   0   1089,1   -     1481,4   1481,4   2563   1719   CTTCACATTCCGTGTGTTSpC^spC   0   1089,1   -       1432,1   23   HLAA   2565   1719   CTTCACATTCCGTGTGTTSpC^spC   0   1089,1   -	15	HLAA_2384_2r20	CTSGTCCCAATACTCAGspGspC^spC						
18   HLAA 2882   120   CYGGTCCAATACTCCGSpGspC^spC   0   1473,4   -   1776,6   -   18   HLAA 2872   1719   CTTCATATTCCGTGTCTCSpC^spT   0   1089,1   -   1392,3   1432,4   120   HLAA 2862   1r19   CTTCACWTTCCGTGTCTCSpC^spT   0   1089,1   -   1392,3   1432,4   1411,4   1457,3   1411,4   1457,3   1411,4   1427,4   1411,4   2822   1720   ACACGGCAATGCCGTGTCTGSpC*spT   0   1089,1   -   1392,3   1432,4   1411,4   1427,4   1411,4   2922   1720   ACACGGCACTCACAGSpAspG*spC   0   1089,1   -   1432,1   1411,4   1441,3   1441,3   1441,4   1457,3   -   1481,4   1457,3   -     1481,4   1481,4   1481,4   1481,4   1481,4   1481	16	HLAA_2385_2r20	CYGGTCCCAATACTCCGspGspC^spC_						
19   HLAA   2581   1719   CITCATATTCCGTGTCTCSpC^spT   0   1089,1   - 1392,3   1432,4	17	HLAA_2386_2r20	CMGGTCCCAATACTCCGspGspC^spC			<u> </u>			<del></del> -
19	18	HLAA_2387_2r20	CYCGTCCCAATACTCCGspGspC^spC	10	14/3,4		1770,0		
19				1_	40004	ļ	1202 3	1432 4	
20 HLAA 2563 1r19 CTTCACATKCCGTGTCTGSpC^spA 0 1138,1 - 1481,4 5 22 HLAA 2564 1r19 CTTCACTTTCCGTGTGTTSpC^spC 0 1089,1 - 1432,1 23 HLAA 2565 1r19 CYTCACATTCCGTGTGTTSpC^spC 0 1089,1 - 1432,1 24 HLAA 2566 1r19 CYTCACATTCCGTGTGTTSpC^spC 0 1089,1 - 1432,1 24 HLAA 2566 1r19 CYTCACATTCCGTGTGTCSpC^spC 0 1074,1 - 1377,3 1417,4 25 HLAA 2567 1r19 CTTCASTTGCCGTGTCTCSpC^spC 0 1074,1 - 1377,3 1417,4 26 HLAA 2568 1r19 CTTCASTTGCCGTGTCTCSpC^spC 0 1074,1 - 1377,3 1417,4 26 HLAA 2568 1r19 CTTCAGTKCCGTGTCTCSpC^spC 0 1074,1 - 1377,3 1417,4 26 HLAA 2568 1r19 CTTCAGTKCCGTGTCTCSpC^spC 0 1074,1 - 1377,3 1417,4 27,4 1481,4 2581 1r20 ATTGGGACCGGAACACACSpG^spC 0 1154,1 1481,4 1457,3 - 147,4 1481,4 2582 1r20 ATTGGGACCGAGACACACSpG^spC 0 1154,1 1481,4 1457,3 - 147,4 1481,4 2582 1r20 ATTGGGACSAGGAGACACSpG^spC 0 1154,1 1481,4 1457,3 - 147,4 1481,4 2584 1r20 ATTGGGACSAGGAGACACSpG^spC 0 1154,1 1481,4 1457,3 - 147,4 1481,4 2584 1r20 ATTGGGACSAGGAGACACSpG^spC 0 1154,1 1481,4 1457,3 - 147,4 1481,4 2584 1r20 ATTGGGACSAGGAGACACSpG^spC 0 1154,1 1481,4 1457,3 - 147,4 1481,4 2584 1r20 ATTGGGACSAGGAGACACSpG^spC 0 1154,1 1481,4 1457,3 - 147,4 1481,4 2584 1r20 ATTGGGACSAGGAGACACSpG^spC 0 1154,1 1481,4 1457,3 - 147,4 1481,4 2584 1r20 ATTGGGACSAGGAGACAGSpG^spC 0 1154,1 1481,4 1457,3 - 147,4 1481,4 2584 1r20 ATTGGGACSAGGAGACAGSpG^spC 0 1194,1 152,1,4 - 1427,4 144,4	19	HLAA_2561_1r19	CTTCATATTCCGTGTCTCspC*sp1			<u> </u>			
Thiam   255	20	HLAA_2562_1r19	CTTCACWTTCCG1G1C1CspC*sp1			<u> </u>			
22 HILAA 2565 1r19 CYTCACATTCCGTGTGTTspC*spC 0 1089,1 1432,1 24 HLAA 2566 1r19 CTTCACATTCCGTGTGTCTCSpC*spC 0 1074,1 - 1377,3 1417,4 25 HLAA 2567 1r19 CTTCACATTCCGTGTCTCSpC*spC 0 1074,1 - 1377,3 1417,4 26 HLAA 2568 1r19 CTTCACATTCCGTGTCTCSpC*spC 0 1074,1 - 1377,3 1417,4 26 HLAA 2568 1r19 CTTCAGTTKCCGTGTCTCSpC*spC 0 1074,1 - 1377,3 1417,4 26 HLAA 2568 1r19 CTTCAGTTKCCGTGTCTCSpC*spC 0 1074,1 - 1377,3 1417,4 26 HLAA 2681 1r20 ATTGGGACCGGAACACACACSpG*spC 0 1074,1 - 1377,3 1417,4 27 141,4 2681 1r20 ATTGGGACCTGCAGACACACACSpG*spC 0 1154,1 1481,4 1457,3 - 29 HLAA 2682 1r20 ATTGGGACSAGGAGACACSpG*spC 0 1154,1 1481,4 1457,3 - 31 HLAA 2684 1r20 ATTGGGACSAGGAGACACSpG*spC 0 1154,1 1481,4 1457,3 - 32 HLAA 2684 1r20 ATTGGGACSGGAGACACSpG*spC 0 1154,1 1481,4 1457,3 - 32 HLAA 2685 1r20 ATTGGGACSAGGAGACACSpG*spC 0 1194,1 1521,4 33 HLAA 2701 1r19 CTGTGACTGGGCCTTCSpA*spC - 14 1084,1 1411,4 - 1427,4 35 HLAA 2702 1r19 CTGTGACTGGGCCTTCSpA*spC - 14 1084,1 1411,4 - 1427,4 35 HLAA 2703 1r19 CTGTGACTGGGCCTTCSpA*spC - 14 1084,1 1411,4 - 1427,4 36 HLAA 2821 1r20 ACACGGAATGTGAAGGCCSpC*spA 0 1098,1 - 1401,3 1441,3 38 HLAA 2822 1r20 ACACGGAATGTGAAGGCCSpC*spA 0 1098,1 - 1401,3 1441,3 39 HLAA 2822 1r20 ACACGGAATGTGAAGGCCSpC*spA 0 1098,1 - 1401,3 1441,3 39 HLAA 2823 1r20 ACACGGAATGTGAAGGCCSpC*spA 0 1098,1 - 1401,3 1441,3 40 HLAA 2921 2r20 TGAAGGCCACTCACAGSpASpG*spT 0 1098,1 - 1401,3 1441,3 40 HLAA 2923 2r20 TGAAGGCCACTCACAGSpASpG*spT 0 1098,1 - 1401,3 1441,3 40 HLAA 2923 2r20 TGAAGGCCCACTCACAGSpASpG*spT 0 1488,4 - 1801,6 - 1932,7 41 HLAA 2922 2r20 TGAAGGCCCACTCACAGSpASpG*spT 0 1488,4 - 1775,6 1815,7 45 HLAA 2923 2r20 TGAAGGCCCACTCACAGSpASpC*spT 0 1427,4 - 1775,6 1815,7 45 HLAA 2923 2r20 TGAAGGCCCACTCACAGSpASpC*spT 0 1427,4 - 1775,6 1815,7 45 HLAA 3684 1r20 TCACACCATCCAGGTAATSpG*spT 0 1441,1 1471,6 1447,1 1487,4 48 HLAA 3684 1r20 TCACACCATCCAGGTAATSpG*spT 0 1144,1 1471,6 1447,1 1487,4 49 HLAA 3684 1r20 TCACACCATCCAGGTAATSpG*spT 0 1144,1 1471,6 1447,1 1487,4 49 HLAA 3684 1r20 TCACACCATCCAGGTAATSpG*spT 0 1144,1 1471,6 1447,1 1487,4 49 HLAA 368	21	HLAA_2563_1r19	CTTCACATKCCGTGTCTGspC^spA			ļ			
23 HLAA 2566 1r19 CTTCAGRTTCCGTGTCTCspC*spC	22	HLAA_2564_1r19	CTTCACITICCGIGIGISPU*spC			<del></del>			
26 HILAA 2567 1r19 CTTCASTTGCCGTGTCTCspC*spC	23	HLAA_2565_1r19	CYTCACATICCGIGIGIIspC*spC			<del> </del>	1377 3		
26 HLAA 2568 1r19 CTTCAGTTKCCGTGTCTCspC*spC 0 1074,1 - 1377,3 1417,4  28 HLAA 2681 1f20 ATTGGGACCGGAACACACspG*spC 0 1154,1 1481,4 1457,3 -  29 HLAA 2682 1f20 ATTGGGACCTGCAGACACSpG*spG 0 1154,1 1481,4 1457,3 -  30 HLAA 2683 1f20 ATTGGGACSAGGAGACACspG*spG 0 1154,1 1481,4 1457,3 -  31 HLAA 2684 1f20 ATTGGGACSAGGAGACACspG*spG 0 1154,1 1481,4 1457,3 -  32 HLAA 2685 1f20 ATTGGGACSAGGAGACACspG*spG 0 1154,1 1481,4 1457,3 -  32 HLAA 2685 1f20 ATTGGGACSAGGAGACACspG*spG 0 1154,1 1481,4 1457,3 -  33 HLAA 2701 1r19 CTGTGAGTGGGCCTTCspA*spT 0 1194,1 1521,4 -  34 HLAA 2702 1r19 CTGTGACTGGGCCTTCspA*spT 0 1113,1 1440,4 -  35 HLAA 2703 1r19 CTGTGACTGGGCCTTCspA*spC -14 1084,1 1411,4 - 1427,4   36 HLAA 2821 1f20 ACACGGAATGTGARGGGCspC*spA 0 1098,1 - 1401,3 1441,3  37 HLAA 2822 1f20 ACACGGAATGTGAAGGCCspC*spA 0 1098,1 - 1401,3 1441,3  38 HLAA 2823 1f20 ACACGGCAWGTGAAGGCCspC*spA 0 1098,1 - 1401,3 1441,3  38 HLAA 2823 1f20 ACACGGAATGTGAAGGCCspC*spA 0 1098,1 - 1401,3 1441,3  40 HLAA 2825 1f20 ACACGGAATGTGAAGGCCspC*spA 0 1098,1 - 1401,3 1441,3  41 HLAA 2921 2f20 TGAAGGCCACTCACAGspAspG*spA 0 1098,1 - 1401,3 1441,3  42 HLAA 2922 2f20 TGAAGGCCACTCACAGspAspG*spT 0 1488,4 - 1801,6 -  44 HLAA 2922 2f20 TGAAGGCCACTCACAGspAspG*spT 0 1488,4 - 1801,6 -  45 HLAA 2923 2f20 TGAAGGCCCACTCACAGspAspG*spT 0 1427,4 - 1775,6 1815,7  46 HLAA 2923 2f20 TGAAGGCCCACTCACAGspAspC*spT 0 1427,4 - 1775,6 1815,7  47 HLAA 2923 2f20 TGAAGGCCCACTCACAGspAspC*spT 0 1427,4 - 1775,6 1815,7  48 HLAA 3681 1f20 TCACACCATCCAGGATAATspG*spT 0 1441,1 1471,6 1447,1 1487,4  49 HLAA 3683 1f20 TCACACCATCCAGATAATspG*spT 0 1144,1 1471,6 1447,1 1487,4  49 HLAA 3684 1f20 TCACACCATCCAGATAATspG*spT 0 1144,1 1471,6 1447,1 1487,4  49 HLAA 3684 1f20 TCACACCATCCAGATAATspG*spT 0 1144,1 1471,6 1447,1 1487,4  49 HLAA 3684 1f20 TCACACCATCCAGATGATSpG*spT 0 1144,1 1471,6 1447,1 1487,4  49 HLAA 3684 1f20 TCACACCATCCAGATGATSpG*spT 0 1144,1 1471,6 1447,1 1487,4  49 HLAA 3684 1f20 TCACACCATCCAGATGATSpG*spT 0 1144,1 1471,6 1447,1 1487,4  49 HLAA 3684 1f20 TCACACCATCCAGATGATSpG*spT 0 1144,1 1471,6 1447,1 1	24	HLAA_2566_1r19	CTTCACRITCCGTGTCTCspc-spc			<del></del> -			
28 HLAA 2861 1f20 ATTGGGACCGGAACACACACSpG^spG 0 1154,1 1481,4 1457,3 - 29 HLAA 2682 1f20 ATTGGGACCTGCAGACACSpG^spG 0 1154,1 1481,4 1457,3 - 30 HLAA 2683 1f20 ATTGGGACSAGGAGACACSpG^spG 0 1154,1 1481,4 1457,3 - 31 HLAA 2683 1f20 ATTGGGACSAGGAGACACspG^spG 0 1154,1 1481,4 1457,3 - 32 HLAA 2684 1f20 ATTGGGACSAGGAGACACspG^spG 0 1154,1 1481,4 1457,3 - 32 HLAA 2685 1f20 ATTGGGACSAGGAGACACspG^spG 0 1154,1 1481,4 1457,3 - 33 HLAA 2701 1r19 CTGTGAGTGGGCCTTCspA^spT 0 1194,1 1521,4 34 HLAA 2702 1r19 CTGTGAGTGGGCCTTCspA^spT 0 1113,1 1440,4 35 HLAA 2703 1r19 CTGTGAGTGGGCCTTCspA^spC -14 1084,1 1411,4 - 1427,4  36 HLAA 2821 1f20 ACACGGAATGTGARGGGCspC^spA 0 1098,1 - 1401,3 1441,3 37 HLAA 2822 1f20 ACACGGAATGTGARGGGCspC^spA 0 1098,1 - 1401,3 1441,3 38 HLAA 2823 1f20 ACACGGAACGTGAAGGCCspC^spA 0 1098,1 - 1401,3 1441,3 39 HLAA 2824 1f20 ACACGGAACGTGAAGGCCspC^spA 0 1098,1 - 1401,3 1441,3 40 HLAA 2825 1f20 ACACGGAACGTGAAGGCCspC^spA 0 1098,1 - 1401,3 1441,3 41 HLAA 2821 1f20 ACACGGAACGTGAAGGCCspC^spA 0 1098,1 - 1401,3 1441,3 41 HLAA 2821 1f20 ACACGGAACGTGAAGGCCspC^spA 0 1098,1 - 1401,3 1441,3 42 HLAA 2822 1f20 TGAAGGCCCACTCACAGspAspG^spT -14 1488,4 - 1801,6 - 1401,3 1441,3 41 HLAA 2922 2f20 TGAAGGCCCACTCACAGspAspT^spT 0 1488,4 - 1801,6 - 1831,7 42 HLAA 2923 2f20 TGAAGGCCCACTCACAGspAspT^spT 0 1488,4 - 1801,6 - 1932,9 114,4 144,4	25	HLAA_2567_1r19	CTTCASTTGCCGTGTCTCspC-spC						
29 HLAA 2881 1120 ATTGGGACCTGCAGACACSpG*spG 0 1154,1 1481,4 1457,3 - 30 HLAA 2683 1f20 ATTGGGACCTGCAGACACSpG*spG 0 1154,1 1481,4 1457,3 - 31 HLAA 2684 1f20 ATTGGGACSAGGAGACACSpG*spG 0 1154,1 1481,4 1457,3 - 32 HLAA 2685 1f20 ATTGGGACSAGGAGACACSpG*spG 0 1154,1 1481,4 1457,3 - 32 HLAA 2685 1f20 ATTGGGACSAGGAGACACSpG*spG 0 1194,1 1521,4 - 33 HLAA 2701 1r19 CTGTGAGTGGGCCTTCSpA*spT 0 1113,1 1440,4 - 34 HLAA 2702 1r19 CTGTGACTGGGCCYTCSpA*spC -14 1084,1 1411,4 - 1427,4 35 HLAA 2703 1r19 CTGTGAGTGGGCCTTCSpA*spC -14 1084,1 1411,4 - 1427,4 36 HLAA 2821 1f20 ACACGGAATGTGARGGGCSpC*spA 0 1098,1 - 1401,3 1441,3 37 HLAA 2822 1f20 ACACGGAATGTGAAGGCCSpC*spA 0 1098,1 - 1401,3 1441,3 38 HLAA 2823 1f20 ACACGGAACGTGAAGGCCSpC*spA 0 1098,1 - 1401,3 1441,3 39 HLAA 2824 1f20 ACACGGAACGTGAAGGCCSpC*spA 0 1098,1 - 1401,3 1441,3 40 HLAA 2825 1f20 ACACGGAACGTGAAGGCCSpC*spA 0 1098,1 - 1401,3 1441,3 41 HLAA 2921 2f20 TGAAGGCCCACTCACAGSpASpC*spA 0 1098,1 - 1401,3 1441,3 42 HLAA 2922 2f20 TGAAGGCCCACTCACAGSpASpC*spT 0 198,1 - 1401,3 1441,3 43 HLAA 2922 2f20 TGAAGGCCCACTCACAGSpASpC*spT 0 1488,4 - 1801,6 - 41 HLAA 2924 2f20 TGAAGGCCCACTCACAGSpASpC*spT 0 1488,4 - 1801,6 - 1831,7 43 HLAA 2925 2f20 TGAAGGCCCACTCACAGSpASpC*spT 0 1427,4 - 1775,6 1815,7 45 HLAA 3681 1f20 TCACACCATCCAGASPASpC*spT 0 1427,4 - 1775,6 1815,7 46 HLAA 3682 1f20 TCACACCATCCAGASPASPC*spT 0 1427,4 - 1775,6 1815,7 47 HLAA 3682 1f20 TCACACCATCCAGATAATSpG*spT 0 1144,1 1471,6 1447,1 1487,4 48 HLAA 3683 1f20 TCACACCATCCAGATAATSpG*spT 0 1144,1 1471,6 1447,1 1487,4 49 HLAA 3684 1f20 TCACACCATCCAGATGATSpG*spT 0 1144,1 1471,6 1447,1 1487,4	26	HLAA_2568_1r19	CTICAGTIKCCGTGTCTCspc-spc	╁╩	10/4,1	<del> </del>	.0,0	····	
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33 HLAA 2701 1r19 CTGTGAGTGGGCCTTCspA^spT		HLAA_2684_1120	ATTOCACEAGGAGACAGERGAERG				-	-	-
35 HLAA 2702 1r19 CTGTGACTGGGCCYTCspA*spC -14 1084,1 1411,4 - 1427,4 35 HLAA 2703 1r19 CTGTGAGTGGSCCTTCspA*spC -14 1084,1 1411,4 - 1427,4 36 HLAA 2821 1f20 ACACGGAATGTGARGGGCspC*spA 0 1098,1 - 1401,3 1441,3 37 HLAA 2822 1f20 ACASGGAAGTGAAGGCCspC*spA 0 1098,1 - 1401,3 1441,3 38 HLAA 2823 1f20 ACACGGCAWGTGAAGGCCspC*spA 0 1098,1 - 1401,3 1441,3 39 HLAA 2824 1f20 ACACGGAACGTGAAGGCCspC*spA 0 1098,1 - 1401,3 1441,3 40 HLAA 2825 1f20 ACACGGAACGTGAAGGCCspC*spA 0 1098,1 - 1401,3 1441,3 41 HLAA 2921 2f20 TGAAGGCCCACTCACAGspAspG*spT -14 1498,4 - 1801,6 - 42 HLAA 2922 2f20 TGAAGGCCCACTCACAGspAspG*spT 0 1488,4 1831,7 43 HLAA 2922 2f20 TGAAGGCCCACTCACAGspAspC*spT 0 1488,4 1831,7 44 HLAA 2924 2f20 TGAAGGSCCACTCACAGspAspC*spT 0 1427,4 - 1775,6 1815,7 45 HLAA 2925 2f20 TGAAGGCCCAGTCACAGspAspC*spT 0 1427,4 - 1775,6 1815,7 45 HLAA 3681 1f20 TCACACCATCCAGATAATspG*spC 0 1129,1 1456,4	32	HLAA 2685 1120	ATTGGGACGAGGAGACAGSPC SPC	╅╸	1.0.,.	1			
35 HLAA 2702 1r19 CTGTGACTGGGCCYTCspA^spC -14 1084,1 1411,4 - 1427,4 35 HLAA 2703 1r19 CTGTGACTGGGCCYTCspA^spC -14 1084,1 1411,4 - 1427,4  36 HLAA 2821 1f20 ACACGGAATGTGARGGGCspC^spA 0 1098,1 - 1401,3 1441,3 37 HLAA 2822 1f20 ACACGGAAGTGAAGGCCspC^spA 0 1098,1 - 1401,3 1441,3 38 HLAA 2823 1f20 ACACGGCAWGTGAAGGCCspC^spA 0 1098,1 - 1401,3 1441,3 39 HLAA 2824 1f20 ACACGGCAWGTGAAGGCCspC^spA 0 1098,1 - 1401,3 1441,3 40 HLAA 2825 1f20 ACACGGAACGTGAAGGCCspC^spA 0 1098,1 - 1401,3 1441,3 41 HLAA 2921 2f20 TGAAGGCCCACTCACAGspAspG^spT -14 1498,4 - 1801,6 - 42 HLAA 2922 2f20 TGAAGGCCCACTCACAGspAspG^spT 0 1488,4 - 1831,7 43 HLAA 2922 2f20 TGAAGGCCCACTCACAGspAspC^spT 0 1488,4 - 1831,7 43 HLAA 2923 2f20 TGAAGGSCCACTCACAGspAspC^spT 0 1427,4 - 1775,6 1815,7 45 HLAA 2925 2f20 TGAAGGCCCAGTCACAGspAspC^spT 0 1427,4 - 1775,6 1815,7 45 HLAA 3681 1f20 TCACACCATCCAGATAATspG^spC 0 1129,1 1456,4 46 HLAA 3681 1f20 TCACACCATCCAGATAATspG^spT 0 1427,4 - 1775,6 1815,7 47 HLAA 3682 1f20 TCACACCATCCAGATAATspG^spT 0 1144,1 1471,6 1447,1 1487,4 48 HLAA 3683 1f20 TCACACCATCCAGATAATspG^spT 0 1144,1 1471,6 1447,1 1487,4 49 HLAA 3684 1f20 TCACACCATCCAGATGATSpG^spT 0 1144,1 1471,6 1447,1 1487,4 49 HLAA 3684 1f20 TCACACCCTCCAGATGATSpG^spT 0 1144,1 1471,6 1447,1 1487,4	1-00	1111 44 0704 4-40	CTCTCACTCCCCCTTCenAAenT	10	1113.1	1440.4		-	-
35 HLAA 2703 1r19 CTGTGAGTGGSCCTTCspA^spC -14 1084,1 1411,4 - 1427,4  36 HLAA 2821 1f20 ACACGGAATGTGARGGGCspC^spA	33	HLAA 2701 1719	CTGTGACTGGGCCYTCspA^spC				-	1427,4	1402,4
36 HLAA 2821 1f20 ACACGGAATGTGARGGGCspC^spA 0 1098,1 - 1401,3 1441,3 37 HLAA 2822 1f20 ACASGGAAAGTGAAGGCCspC^spA 0 1098,1 - 1401,3 1441,3 38 HLAA 2823 1f20 ACACGGCAWGTGAAGGCCspC^spA 0 1098,1 - 1401,3 1441,3 39 HLAA 2824 1f20 ACACGGAACGTGAAGGCCspC^spA 0 1098,1 - 1401,3 1441,3 40 HLAA 2825 1f20 ACACGGAACGTGAAGGCCspC^spA 0 1098,1 - 1401,3 1441,3 41 HLAA 2921 2f20 TGAAGGCCCACTCACAGSpAspG^spT -14 1498,4 - 1801,6 - 42 HLAA 2922 2f20 TGAAGGCCCACTCACAGSpAspG^spT 0 1488,4 1831,7 43 HLAA 2923 2f20 TGAAGGCCCACTCACAGSpAspC^spT 0 1589,6 - 1932,9 44 HLAA 2924 2f20 TGAAGGCCCACTCACAGSpAspC^spT 0 1427,4 - 1775,6 1815,7 45 HLAA 2925 2f20 TGAAGGCCCASTCACAGSpAspC^spT 0 1427,4 - 1775,6 1815,7 46 HLAA 3681 1f20 TCACACCATCCAGATAATspG^spC 0 1129,1 1456,4 47 HLAA 3682 1f20 TCACACCATCCAGMTAATspG^spT 0 1144,1 1471,6 1447,1 1487,4 48 HLAA 3683 1f20 TCACACCATCCAGMTAATspG^spT 0 1144,1 1471,6 1447,1 1487,4 49 HLAA 3684 1f20 TCACACCCTCCAGATGATSpG^spT 0 1144,1 1471,6 1447,1 1487,4	34	HLAA 2702 1719	CTGTGAGTGGGCCTTCspA spC					1427,4	1402,4
36 HLAA 2822_1f20 ACASGGAAAGTGAAGGCCspC^spA	35	HLAA 2703 Tris	C1616A61666667163671366	1	1	<b> </b>			
36 HLAA 2822_1f20 ACASGGAAAGTGAAGGCCspC^spA	30	111 AA 2024 4520	ACACGGAATGTGARGGGCsnC^snA	0	1098.1	-	1401,3	1441,3	
37 HLAA 2822 1120 ACACGCAWGTGAAGGCCspC^spA 0 1098,1 - 1401,3 1441,3 39 HLAA 2824 1f20 ACACGGAACGTGAAGGCCspC^spA 0 1098,1 - 1401,3 1441,3 40 HLAA 2825 1f20 ACACGGAATRTGAAGGCCspC^spA 0 1098,1 - 1401,3 1441,3 1441,3 40 HLAA 2921 2f20 TGAAGGCCCACTCACAGspAspG^spT -14 1498,4 - 1801,6 - 42 HLAA 2922 2f20 TGAAGGCCCACTCACAGspAspG^spT 0 1488,4 - 1831,7 43 HLAA 2923 2f20 TGAAGGSCCACTCACAGspAspT^spT 0 1589,6 - 1932,9 144 HLAA 2924 2f20 TGAAGGSCCACTCACAGspAspT^spT 0 1427,4 - 1775,6 1815,7 145 HLAA 2925 2f20 TGAAGGCCCAGTCACAGspAspC^spT 0 1427,4 - 1775,6 1815,7 145 HLAA 3681 1f20 TCACACCATCCAGATAATspG^spC 0 1129,1 1456,4 - 1775,6 1815,7 145 HLAA 3682 1f20 TCACACCATCCAGATAATspG^spT 0 1144,1 1471,6 1447,1 1487,4 1487,4 149 HLAA 3683 1f20 TCACACCSTCCAGAGGATSpG^spT 0 1144,1 1471,6 1447,1 1487,4 149 HLAA 3684 1f20 TCACACCVTCCAGATGATSpG^spT 0 1144,1 1471,6 1447,1 1487,4 149 HLAA 3684 1f20 TCACACCVTCCAGATGATSpG^spT 0 1144,1 1471,6 1447,1 1487,4 149 HLAA 3684 1f20 TCACACCVTCCAGATGATSpG^spT 0 1144,1 1471,6 1447,1 1487,4 149 HLAA 3684 1f20 TCACACCVTCCAGATGATSpG^spT 0 1144,1 1471,6 1447,1 1487,4 149 HLAA 3684 1f20 TCACACCVTCCAGATGATSpG^spT 0 1144,1 1471,6 1447,1 1487,4 149 HLAA 3684 1f20 TCACACCVTCCAGATGATSpG^spT 0 1144,1 1471,6 1447,1 1487,4 149 HLAA 3684 1f20 TCACACCVTCCAGATGATSpG^spT 0 1144,1 1471,6 1447,1 1487,4 149 HLAA 3684 1f20 TCACACCVTCCAGATGATSpG^spT 0 1144,1 1471,6 1447,1 1487,4 149 HLAA 3684 1f20 TCACACCVTCCAGATGATSpG^spT 0 1144,1 1471,6 1447,1 1487,4 149 HLAA 3684 1f20 TCACACCVTCCAGATGATSpG^spT 0 1144,1 1471,6 1447,1 1487,4 149 HLAA 3684 1f20 TCACACCVTCCAGATGATSpG^spT 0 1144,1 1471,6 1447,1 1487,4 1487,4 149 HLAA 3684 1f20 TCACACCVTCCAGATGATSpG^spT 0 1144,1 1471,6 1447,1 1487,4 1487,4 149 HLAA 3684 1f20 TCACACCVTCCAGATGATSpG^spT 0 1144,1 1471,6 1447,1 1487,4 1487,4 149 HLAA 3684 1f20 TCACACCVTCCAGATGATSpG^spT 0 1144,1 1471,6 1447,1 1487,4 149 HLAA 3684 1f20 TCACACCVTCCAGATGATSpG^spT 0 1144,1 1471,6 1447,1 1487,4 149 HLAA 3684 1f20 TCACACCVTCCAGATGATSpG^spT 0 1144,1 1471,6 1447,1 1487,4 149 HLAA 3684 1f20 TCACACCACACACACACAC	30	HI AA 2021 1120	ACASGGAAAGTGAAGGCCsnC^snA			1.			-
39 HLAA 2824 1f20 ACACGGAACGTGAAGGCCspC^spA 0 1098,1 - 1401,3 1441,3 40 HLAA 2825 1f20 ACACGGAATRTGAAGGCCspC^spA 0 1098,1 - 1401,3 1441,3 1441,3	37	HLAA_2022_1120	ACACGCAWGTGAAGGCCspCAspA			-	1401,3	1441,3	-
40 HLAA 2825 1f20 ACACGGAATRTGAAGGCCspC^spA 0 1098,1 - 1401,3 1441,3  41 HLAA 2921 2f20 TGAAGGCCCACTCACAGspAspG^spT -14 1498,4 - 1801,6 -  42 HLAA 2922 2f20 TGAAGGCCCACTCACAGspAspC^spT 0 1488,4 - 1831,7  43 HLAA 2923 2f20 TGAAGGSCCACTCACAGspAspT^spT 0 1589,6 - 1932,9  44 HLAA 2924 2f20 TGARGGCCCAGTCACAGspAspC^spT 0 1427,4 - 1775,6 1815,7  45 HLAA 2925 2f20 TGAAGGCCCASTCACAGspAspC^spT 0 1427,4 - 1775,6 1815,7  46 HLAA 3681 1f20 TCACACCATCCAGATAATspG^spC 0 1129,1 1456,4  47 HLAA 3682 1f20 TCACACCATCCAGATAATspG^spT 0 1144,1 1471,6 1447,1 1487,4  48 HLAA 3683 1f20 TCACACCSTCCAGAGGATSpG^spT 0 1144,1 1471,6 1447,1 1487,4  49 HLAA 3684 1f20 TCACACCVTCCAGATGATSpG^spT 0 1144,1 1471,6 1447,1 1487,4	5 38	HLAA 2023_1120	ACACGGAACGTGAAGGCCspC^spA			-	1401,3	1441,3	-
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43 HLAA 2923 Z120 TGAAGGCCAGTCACAGSPASpT 0 1427,4 - 1775,6 1815,7  44 HLAA 2924 Z120 TGAAGGCCCAGTCACAGSPASpC^spT 0 1427,4 - 1775,6 1815,7  45 HLAA 2925 Z120 TGAAGGCCCASTCACAGSPASpC^spT 0 1427,4 - 1775,6 1815,7  46 HLAA 3681 1120 TCACACCATCCAGATAATSpG^spC 0 1129,1 1456,4	42	ILLAA 2022 2520	TGAAGGSCCACTCACAGenAenT^enT			-	-	1932,9	
44 HLAA 2924 ZIZB TCARCCCCATCACAGSPASPC^SpT 0 1427,4 - 1775,6 1815,7  45 HLAA 2925 Zf20 TGAAGGCCCASTCACAGSPASPC^SpT 0 1427,4 - 1775,6 1815,7  46 HLAA 3681 1f20 TCACACCATCCAGATAATSpG^SpC 0 1129,1 1456,4	43	ITLAA 2024 2520	TCARCCCCAGTCACACenAenCAenT			<del>  -</del>	1775,6		
46 HLAA 3681 1f20 TCACACCATCCAGATAATspG^spC 0 1129,1 1456,4 47 HLAA 3682 1f20 TCACACCATCCAGMTAATspG^spT 0 1144,1 1471,6 1447,1 1487,4 48 HLAA 3683 1f20 TCACACCSTCCAGAGGATSpG^spT 0 1144,1 1471,6 1447,1 1487,4 49 HLAA 3684 1f20 TCACACCVTCCAGATGATspG^spT 0 1144,1 1471,6 1447,1 1487,4		ITLAA 2025 2520	TGAAGGCCCASTCACAGenAenChenT						-
47       HLAA 3682 1f20 TCACACCATCCAGMTAATspG^spT       0 1144,1 1471,6 1447,1 1487,4         48       HLAA 3683 1f20 TCACACCSTCCAGAGGATspG^spT       0 1144,1 1471,6 1447,1 1487,4         49       HLAA 3684 1f20 TCACACCVTCCAGATGATspG^spT       0 1144,1 1471,6 1447,1 1487,4	45	HLAA_2925_2120	I GANGGOOMS (CNONGSPASPO SP)	┪	1	1	<b>T</b>	1.	
47       HLAA 3682 1f20       TCACACCATCCAGMTAATspG^spT       0       1144,1       1471,6       1447,1       1487,4         48       HLAA 3683 1f20       TCACACCSTCCAGAGGATspG^spT       0       1144,1       1471,6       1447,1       1487,4         49       HLAA 3684 1f20       TCACACCVTCCAGATGATspG^spT       0       1144,1       1471,6       1447,1       1487,4	م ا	N AA 0004 4500	TCACACCATCCAGATAATanGAanC	10	1129.1	1456.4	1 -	1 -	-
47 HLAA 3682 1120 TCACACCATCOAGMTSTSPS 557 48 HLAA 3683 1f20 TCACACCSTCCAGAGGATspG^spT 0 1144,1 1471,6 1447,1 1487,4 49 HLAA 3684 1f20 TCACACCVTCCAGATGATspG^spT 0 1144,1 1471,6 1447,1 1487,4	46	HLAA 3681 1120	TCACACCATCCAGATAATSPG SPO			1471:6	1447.1	1487,4	1462,
49 HLAA 3684_1f20 TCACACCVTCCAGATGATspG^spT 0 1144,1 1471,6 1447,1 1487,4	47	HLAA 3682 1120	TCACACCSTCCAGAGGATenGAenT			1471.6	1447.1		
43 MEAA 3004_1120 107.04.00 103.		HLAA_3683_1120	TOACACOVITCOAGATGATERCACAT			1471 6	1447.1	1487,4	
, , , , , , , , , , , , , , , , , , ,	49	HLAA_3684_1120	TOMOMOGN TOOMGATGATGAG SPT	┵	† · · · · · · · · · · · · · · · · · · ·	1	<del>                                     </del>	1	T
50 HLAA_3961_2r20 GCTGGTACCCGCGGAGspGspA^spG	1 ==	1111 AA 2004 0 00	CCTGGTACCCGCGCAGanGanAAanG	1	1537.4	<del>                                     </del>	<del>  .</del>	1880,7	

BNSDOCID: <WO\_\_\_\_2005052189A2\_I\_>

r	E4 1	ULAA 2062 2r20	GCCGGTACCCGCGGAGspTspA^spA	0	1496,4	- 1	- 1	1839,7	-
ŀ	51	HLAA 3902_2120	GGTGGTACCCGYGCAGspGspA^spA	0	1496,4	-	- 1	1839,7	
ł	52	HLAA 3903 2120	GGTGGTACCCGCAGAGspGspA^spA	0	1521,5		-	1864,8	1839,7
1	53	HLAA 3904 2120	GTTCATACCCGCGGAGspGspA^spA	0	1521,5		- 1	1864,8	1839,7
ŀ	54	HLAA 3965_2120	GSTGGTACCCGCGGAGspGspA^spA	0	1521,5	-	-	1864,8	
	55	HLAA 3966_2120	GCCGGTACCCGCGGAGspGspA^spA	0	1521,5				1839,7
ŀ	56	HLAA_3967_2120	Оссостиссоссанизразри орг.						
Į		111 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	CGCTTCCTCCGCGGGTATspG^spA	0	1153,1	1480,1			- 1
	57	HLAA_4141_1120_	CGCTTCCTCTGCGGGTACspC^spA	ō	1098,1			1441,4	-
1	58	HLAA 4142 1120	CGCTTCCTGCGCGGGTACspC^spA	ŏ	1098,1		1401,3		-
5	59	HLAA 4143 1120	CGCTTCCTCCACGGGTACspC^spA	ō	1098,1		1401,3		-
1	60	HLAA_4144_1120	CGMTTCCTCCGCGGGTACspC^spA	Ö	1098,1		1401,3		-
1	61	HLAA 4145 1120	CGCCTCCTCCGCGGGTACspC^spA	ō	1098,1		1401,3		-
ı	62	HLAA 4146 1120	CACTTCCTCCGCGGGTACspC^spG	0	1114,1		-	1457,4	-
į	63	HLAA_4147_1120	CGCTTMCTCCGCGGGTACspC^spG	0	1114,1			1457,4	-
	64	HLAA_4148_1120	CGC TWC TCCGCGGGTACspc spc	-	11111				
			CTCCAACACCCCACCTCTenTAenC	0	1206,2				1524,4
	65	HLAA 4531 1r20	GTCCAAGAGCGCAGGTCTspT^spC	ŏ	1191,2			1534,5	1509,4
	66	HLAA_4532_1120	GTCCAAGAGCGCAGGTCCspT^spC	0	1191,2			1534,5	1509,4
10	67	HLAA_4533_1120	GTCCAGGAGCTCAGGTCCspT^spC	<u> </u>	1,01,2				
			CCCCCVCTCCCACTTGTenGenCAenT	0	1463,4	-			1781,6
	68	HLAA_5021_2r20	GGCCGYCTCCCACTTGTspGspC^spT GGCYGCCTCCCACTTGCspGspC^spT	6	1448,4		1751,6	1791.7	1766,6
	69	HLAA 5022 2120	CGGAGTCTCCCACTTGCspGspC^spT	0	1448,4		1751,6	1791.7	1766,6
	70	HLAA 5023 2120	CGGAGTCTCCCACTTGCspGspC spT	-14				1	1737,6
	71	HLAA_5024_2r20	GGCCGCCTCCCACTTGCspGspC^spC		1710,4				
-			ACTOCO ACACTOCOCO CAENTAENG	0	1255,3	1582,6	1558.5	-	1573,5
!	72	HLAA 5271 1120	AGTGGGAGACTCCGCCCAspT^spG	0	1255,3	1582,6		-	1573,5
	73	HLAA_5272_1120	CAAGTGGGAGGCGGYCCAspT^spG CAAGTGGGAGRCGGCCCAspT^spG	Ö	1255,3	1582,6	1558,5	_	1573,5
1.5	74	HLAA 5273 1720	CAAGTGGGAGGCGGCCCTspT^spG	0	1246,3	-	-		1564,5
15	75	HLAA 5274 1120	CAACTCCCAGCCGCCCCGpT^spT	0	1246,3	-	-	1589,6	-
	76	HLAA_5275_1120	CAAGTGGGAGGCGGCCCGspT^spT CAAGTGGGAGGCGGCCCGspT^spC	ō	1231,3	-		1574,5	•
		HLAA 5276_1f20	CAAGTGGGAGGCGGCCMGspT^spG	0	1271,3	1598,6	-	-	1589,5
		HLAA 5277_1f20		0	1271,3	1598,6		-	1589,5
	79	HLAA_5278_1f20	CAAGTGGGAGGCRGCCGGSpT spc	<u> </u>	12: 1,5				
	- 00	111 4 4 5004 4540	GCCCRTGAGGCGGAGCAspG^spC	0	1138,1	1465,4		1481,4	1456,3
	80	HLAA 5391 1119	GYCCATGCGGCGGAGCAspG^spC	0	1138,1	1465,4		1481,4	1456,3
	81	HLAA 5392 1119	GCCGTCGGGCGAGCAspG^spC	Ò	1138,1	1465,4	_	1481,4	1456,3
	82	HLAA 5393 1119	GCCCATGTGGCGGAGCAspG^spC	ō	1138,1	1465,4	-	1481,4	1456,3
20	83	HLAA 5394 1119	GTCCATGCGGCGGAGCAspG^spT	ō	1153,1	-		1496,4	1471,3
	84	HLAA 5395 1119	GCCGTYGGGCGGAGCAspG^spT	ō	1153,1	-	-	1496,4	
	85	HLAA 5396 1119	GCCATGAGGCGGAGCAspG^spT	ō	1153,1	-	-	1496,4	1471,3
	86	HLAA 5397 1119	GCCCWTGTGGCGGAGCAspG^spT	ō	1153,1	-	-	1496,4	1471,3
	87	HLAA 5398 1119	GCCMGTGTGGCGGAGCAspG^spT	tõ	1153,1			1496,4	1471,3
	88	HLAA_5399_1119	GCCMG16166666666666666666666666666666666	<del>                                     </del>		-			
	<u> </u>	10 44 5504 4-00	GCGGAGCCACTCCACGCAspC^spT	10	1113,1	<del></del>	1416,3	-	
	1 8a	ITLAA 5500 4-00	GCGGAGCCCGTCCACGCAspC sp1	10	1113,1	-	1416,3	-	
	90	ITLAA 5592 1720	GCGGAGCCACTCCACGCAspC^spA	Ö	1122,1	<del>  -</del>	-	1465,4	
25	91	HLAA_5593_1720	GCGGAGCCGTCCACTCAspC^spG	0	1138,1		-	-	1456,3
	92	HLAA 5594 1120	GCGGAGCCAGTCCACGCAspC^spG	0	1138,1	-	-	-	1456,3
	93	HLAA_5595_1720	GCGGAGCCMGTCCACGCAspC^spG	10	1138,1		-	-	1456,3
	94	HLAA_5596_1720	GCGGAGCCACTCCACGCAspC*spC	Ö	1098,1	1425,4	-	1441,4	- 1
	95	HLAA 5597 1720	LCCCACCCCTCCACGCAspC spc	tŏ	1098,1	1425,4		1441,4	
	96	HLAA 5598 1720	GCGGAGCCGTCCACGCAspC^spC	tŏ	1178,1	- 1	<del>  -</del>	-	1496,3
	-	HLAA_5599_1r20	GCGGAGCCACTCCACGCAspG^spG	╅	1	1	1	1	
	1		TOCACCOCCYCTCCCTCccCcnA4ccC	10	1537,4	<del> </del>	<del>  -</del>	-	1855,6
	97	HLAA_5711_2f20	TGGAGGCCKGTGCGTGspGspA^spG	10	1537,4	<del>  -</del>	-	<del>  -</del>	1855,6
30	98	JHLAA_5712_2f20	TGGAGGGYGAGTGCGTGspGspA^spG	10	1537,4	<del>                                     </del>	1	-	1855,6
50	99	HLAA_5713_2f20	TGSAGGGCCGGTGCGTGspGspA^spG	1 0	1537,4	1	1 -	-	1855,6
	100	HLAA 5714 2f20	TGGATGSCACGTGCGTGspGspA^spG	1 8	1537,4	1	<del> </del>	1 -	1855,6
-	101	HLAA_5715_2f20	TGGAGGCACSTGCGTGspGspA^spG	1 8		<del></del>	+	1840,7	
	102	HLAA_5716_2f20	TGGAGGCACGTGMGTGspGspA^spC	10		<del>                                     </del>	<del>                                     </del>	1840,7	
	103	HLAA_5717_2f20	TGGAGGCYGGTGCGTGspGspA^spC		1 ,731,7	ــــــــــــــــــــــــــــــــــــــ		4	

TABLE V

					Primer			<del></del>	
1			Coguenes	CT	Masses	Α	С	G	Т
l l	No	Name	Sequence	0	1540,3		1843,7		1858,7
- 1	1	11010_01	CCCACTCCATGAGGCATspTspT^spC	0				1883,8	
	2	HLAB_972_2f20	CCCACTYCATGAGGTATspTspT^spC	5	1540,3		1843,7	1883,8	1858,7
1				00	4450.4	44774	4452.2		4400 2
·	3	HLAB_2061_1f20	CGACGCCGCGAGTCMGAGspG^spA	-28	1150,1	1477,4	1453,3		1468,3
ı	4		CGACGCCACGAGTCCGAGspG^spA	-28	1150,1	1477,4	1453,3	4504.4	1468,3
	5		CGACGCCGCGAGTCCRAGspA^spG	0	1178,1	1505,4		1521,4	
_	6	HLAB_2064_1f20	CGACGCCRCGAGTCCGAGspA^spG	0	1178,1	1505,4		1521,4	
5					40000	4405.4		4 4 4 4 4	
	7		GCCCCTCCTGCTCCACCspC^spA	0	1098,3	1425,4		1441,4	
- 1	8	HLAB_2222_1r19	GCCCCTCYTGCTCTATCspC^spA	0	1098,3	1425,4	<u> </u>	1441,4	
1				<u> </u>	4542.4			40507	
1	9		GGCCGGAGTATTGGGACspGspG^spG .	0	1513,4			1856,7 1840,7	
	10	HLAB_2592_2f20	GGCCGGAGTATTGGGACspGspA^spG	0	1497,4		-		
	11	HLAB_2593_2f20	GGCCGGAGTATTGGGACspCspC^spG	-28	1405,4	40457		1748,7	
	12		GGCCGGAGTATTGGGATspCspG^spG	0	1488,4	1815,7		1831,7	
	13		GGCCGGAGTTTTGGGACspCspG^spG	-28	1445,4	1772,7		1788,7 1788,7	•
10	14		GGCCGGAGCATTGGGACspCspG^spG	-28	1445,4	1772,7 1772,7	<u> </u>	1788,7	
-	15		GGCCGGGATATTGGGACspCspG^spG	-28 -28	1445,4	1772,7	<del></del>	1788,7	
	16		GGCCRGAATATTGGGACspCspG^spG		1445,4		<u> </u>	1788,7	
1	17	HLAB_2599_2f20	GGCGGGMGTATTGGGACspCspG^spG	-28	1445,4	1772,7 1772,7		1788,7	
	18	HLAB_25910_2f20	GGCCTTAGTATTGGGACspCspG^spG	-28	1445,4	1112,1		1700,1	
				<u> </u>	4400.4				1440,3
	19	HLAB_2721_1f20	GGACSGGAGACACGGAAspC^spA	0	1122,1				1440,3
1	20	HLAB_2722_1f20	GGACGRGGAGACACGGAAspC^spA	0	1122,1			1456,4	1440,3
ļ	21	HLAB_2723_1f20	GGACCGGAACACACAGAAspC^spT	0	1113,1		<u> </u>	1450,4	1393,3
	22		GGACCGGAACACACAGACspC^spT	-14	1075,1	4400 4			1090,0
15	23	HLAB_2725_1f20	GGACCGGGAGACACAGAAspG^spT	0	1153,1	1480,4 1431,4	1407,3	1447,4	1422,3
	24		GGACCGGGAGATACAGATspC^spT	0	1104,1	1431,4	1407,3	1447,4	1422,3
	25		GGACCGGGASACACAGATspC^spT	0	1104,1	1431,4	1407,3	1447,4	1422,3
ļ	26	HLAB_2728_1f20	GGACCGGGACACACAGATspC^spT	0	1104,1 1104,1	1431,4	1407,3	1447,4	1422,3
	27	HLAB_2729_1f20	GGACCSGGAGACACAGATspC^spT	<u> </u>	1104,1	1431,4	1401,0	1441,4	1422,0
			OAA GA GOAA GA G	0	1458,3			1801,6	
1	28	HLAB_2921_2f19	CAAGACCAACACACAGspGspC^spT	0	1458,3			1801,6	
1	29	HLAB_2922_2f19	CAAGSCCCAGGCACAGspGspC^spT	-28	1414,3			1757,6	1732,5
	30		CAAGACCAACACACGGspAspC^spT	-28	1414,3		-	1757,6	1732,5
20	31	HLAB_2924_2f19	GAAGGCCTCCGCGCAGspAspC^spT	-28	1414,3			1757,6	1732,5
20	32		CAAGGCCMAGGCACAGspAspC^spT	-28	1414,3			1757,6	1732,5
	33		CAAGSGCCAGGCACAGSPAspC^spT	-28	1414,3			1757,6	1732,5
	34	HLAB_2927_2f19	GAAGACCAACACAGspAspC^spT	-20	1414,3	<del> </del> -	<del> </del>	1.0.,5	1102,0
	<u> </u>		IOCACACACTCACCCACTCAC	0	1528,4	<del> </del>	<del> </del>	1871,7	-
	35	HLAB_3021_2119	GCACAGACTTACAGAGGAGAGAGAGAGAGAGAGAGAGAGA	-28	1493,5	1820,8		1836,8	
		HLAB_30211_2f19	ACACAGACTTACAGAGSpAspG^spA	-28	1537,4	1864,7		1000,0	
	37	HLAB 3022 2119	ACACAGACTTACCGAGspAspG^spG	0	1537,4		<del>                                     </del>		
	38	HLAB_3023_2f19	RCACAGACTGACCGAGspAspG^spG	-28	1481,4		<del></del>	1827,7	
	39	HLAB_3024_2f19	GCACAGACTTACCGAGSpTspG^spA	-28			-	1827,7	
25	40	HLAB_3025_2f19	ACACAGACTTACCGAGspTspG^spA			1811,7		1827,7	
	41	HLAB_3026_2f19	RCACAGACTGACCGAGspTspG^spA	-28 -28	1493,5	1820,8		1836,8	
	42	HLAB_3027_2f19	ACACAGGCTGACCGAGspAspG^spA	-28 -28		1820,8	<del></del>	1836,8	
	43	HLAB_3028_2f19	RCACAGACTGACCGAGspAspG^spA					1836,8	
	44	HLAB_3029_2f19	GCRCAGACTTACCGAGspAspG^spA	-28		1820,8 1820,8	-	1836,8	
	45	HLAB_30210_2f19	ACACRGACTTACCGAGspAspG^spA	-28	1493,5	1020,0	<del> </del>	1000,0	
			DOCUMENTO A CARGO CONTROL A COMMITTE OF THE CO		4442 4	<del> </del> -	<del> </del>	1756,7	
	46	HLAB_3621_2f20	CGGGTCTCACACCCTCCspAspC^spA	-28	1413,4	1794,7	1770,6	1810,7	1785,6
	47	HLAB_3622_2f20	CGGGTCTCACAYCATCCspAspG^spA	-14				1810,7	1785,6
20	48	HLAB_3623_2f20	CGGKTCTCACACCCTCCspAspG^spA	-14	1467,4	1794,7		1810,7	1785,6
30	49	HLAB_3624_2f20	CGGGTCTCACACTTGGCspAspG^spA	-14	1467,4	1794,7	1	1010,7	1801,6
Ī	50	HLAB_3625_2f20	CGGGTCTCACATCATCCspAspG^spG	-14		<u> </u>		1815,7	- 1001,0
İ	51	HLAB_3626_2f20	CGGGTCTCACACCCTCCspAspG^spT	0	1472,4	<u> </u>	<del> </del>	1010,1	
	<b> </b>			<del> </del>	4005 #	<del> </del>	1200 2	1428,4	1403,3
	52	HLAB_3631_1r20	CCCASGTCGCAGCCGTACspA^spT	-28	1085,1	<del>  -</del> -	1388,3		1403,3
	53	HLAB_3632_1r20	CCCABGTCGCAGCCATACspA^spT	-28		<del> </del>	1388,3	1428,4	1403,3
	54	HLAB 3633 1r20	CCCASGTCGCAGCCAAACspA^spT	-28	1085,1	<u></u>	1388,3	1420,4	

55 56	HLAB 3634 1r20	CCCACGTCGCAGCCAGACspA^spT	-28	1085,1			1428,4	1403,3
	HI AB 3635 1r20							
		CCCACGTCGCAGCCGCACspA^spT	-28	1085,1		1388,3	1428,4	1403,3
57	HI AB 3636 1r20	CCCACGTCGCAGCCTTACspA^spT	-28	1085,1			1428,4	1403,3
58	HI AB 3637 1r20	CCCACGTCGCAGCCGTACspG^spT	0	1129,1		1432,3	1472,4	1447,3
59	HLAB 3691 1f20	TCCGGCCCCAKGTCGCAGspC^spC	0	1114,1	1441,4	-	1457,4	1432,3
60	HI AB 3692 1f20	TCGGGCCCCASGTCGCAGspC^spC	0	1114,1	1441,4		1457,4	1432,3
F	1,12,12						1	
55	HI AB 4121 2f20	GGCGCCTCCTCCGCGGGspTspA^spC	-28	1444,4	•	1747,6		<u></u>
56	HI AB 4122 2f20	GGCGCCTCCTCCSCGGGspCspA^spT	0	1472,4	1799,7	-	1815,7	
57	HI AB 4123 2f20	GGCGCYTCCTCCGCGGGspCspA^spT	0	1472,4	1799,7	-	1815,7	
5 58	HI AB 4124 2f20	GGCGTCTCCTCCGCGGTspTspA^spT	0	1462,4	-	1765,6	-	
59	HLAB_4125_2f20	GGCGCCTCCTCCGCGGGspTspA^spT	-14	1473,4	-	1776,6		
1								
60	HI AB 4181 2f20	TCCTCCGCGGGTATGAAspCspA^spG	0	1481,4	1808,7			
61	HI AB 4182 2f20	TCCTCCACGGGTACCACspCspA^spG	0	1457,4		-		1775,6
62	HI AB 4183 2f20	TCCTGCGCGGGTACCACspCspA^spG	0	1457,4	•	-		1775,6
63	HI AB 4184 2f20	TCCTCCGCGGGTACCACspCspA^spG	0	1457,4				1775,6
64	HI AB 4185 2f20	TCCTCTGCGGGTACCACspCspA^spG	0	1457,4	-	-		1775,6
65	HI AB 4186 2f20	TCCTCCGCGGGTACCAGspCspA^spG	0	1497,4	1824,7	1800,6	1840,7	1815,6
	HI AB 4187 2f20	TMCTCCGCGGGTACCGGspCspA^spG	0	1497,4	1824,7	1800,6	1840,7	1815,6
10 66	HI AB 4188 2120	TCCTCCGCGGGTACCAGspCspG^spG	0	1513,4	-		1856,7	
15,	(ICAD_IIIO_AIA							
68	HI AB 4191 2r20	AATCCTTGCCGTCGTAGspGspC^spT	-14	1474,4	1801,7			<u> </u>
69	HI AB 4192 2r20	AATCCTTGCCGTCGTAGspGspC^spA	-28	1469,4	-	-	1812,7	<u> </u>
70	HI AB 4193 2r20	AATTCTTGCCGTCGTAGspGspC^spG	0	1513,4	1840,7	-	1856,7	1831,6
71	НІ АВ 4194 2г20	AATCTTTGCCGTCGTAGspGspC^spG	0	1513,4	1840,7		1856,7	1831,6
72	HLAB 4195 2r20	AATCCTTGCCGTCGYAGspGspC^spG	0	1513,4	1840,7		1856,7	1831,6
. [	1		<u> </u>					4740.4
73	HLAB 4351n 1r20	TCMTTCAGGGCGATGTAAspT^spC	-14	1201,3	<u> </u>	1504,4		1519,4
15 74	HLAB_4352n_1r20	TCGTTCAGGGCGATGTAAspT^spT	0	1230,3	<u> </u>	1533,5		
			<u> </u>	<u> </u>		[		AFCAE
75	HLAB_5271_1f20	CAAGTGGGAGGCGGCCCTspT^spG	0	1246,3		1333		1564,5 1589,5
76	HLAB_5272_1f20	CAAGTKGGAGGCGGCCCGspT^spG	0	1271,3	1598,6	1574,3		1505,5
1			ļ				1481,3	1456,3
77	HLAB_5391_1f20	GGCCCGTGYGGCGGAGCAspG^spC	10	1138,1	4 505 4			1450,5
78	HLAB_5392_1f20	GGCCCGTGTCGCGGAGCAspG^spG	0	1178,1	1505,4			<del>-</del>
79	HLAB_5393_1f20	GGCCCGTGWGGCGGAGCAspG^spG	0	1178,1	1505,4		1496,4	<del></del>
80	HLAB_5394_1f20	GGCCCGTGAGGCGGAGCAspG^spT	0	1153,1	<del></del>	ļ	1430,4	
20			<del> </del> _	44424	<b> </b>		1456,4	
81	HLAB_5591_1r20	GCGGAGCGACTCCACGCAspC^spT	10	1113,1		<u> </u>	1456,4	<del></del>
82	HLAB_5592_1r20	GCGGAGCCACTCCACGCAspC^spT	10	1113,1	<del></del>	ļ	1456,4	
	HLAB_5593_1r20	GCGGAGCCAATCCACGCAspC^spT	0	1113,1	<del> </del>	<del>                                     </del>	- 1430,4	1470,3
84	HLAB_5594_1r20	GCGGAGCCACTCCACGCAspC^spG	10	1152,1	1449,1	1425,3		
85	HLAB_5595_1r20	GCGGAGCGACTCCRCGCAspC^spA	-14	1122,1 1122,1	1449,1	1425,3	<del></del>	
86		GCGGAGCSACTCCACGCAspC^spA	-14 -14		1449,1	1425,3	-	
87	HLAB_5597_1r20	GCGGAGCCCGTCCACGCAspC^spA	1-14	1122,1	1443,1	1720,0	l	
L_	1	OTOGA COTAVOTO COCA COSCASO	10	1154,1	1481,4	<del></del>		-
88	HLAB_5711_1r20	CTCCAGGTAYCTGCGGAGspC^spG	1 6	1114,1	1441,4		-	-
25 89	HLAB_5712_1r20	CTCCAGGTRTCTGCGGAGspC^spC	<del>                                     </del>	<del>                                     </del>	1,44,1,4	<del> ,</del>		
	111 AD 500 4540	ACCTGGAGAACGGGAAGspG^spA	10	1178,1	1505,4		1521,4	-
90	HLAB_583_1r19	MOCTOGAGAACGGGAAAGSPO SPA			1	<del></del>		

TABLE VI

				Masses				1
- I		<b>C</b>	СТ	Primer	Α	С	G	Т
No	Name	Sequence	10	1098,1	<del></del>	1392,3	<del> 1</del>	
1	D1101_1201_	CATTGAAGAAATGACACTspC^spC	0	1230,1	<del></del>	1002,0		1548,5
2	D1/D   140-14-1	CGTTGAAGAAATGACACTspT^spA	10	1113,1	1440,4	1416,3	1456,4	1431,3
3		CATTGAAGAAATGACATTspC^spA	10	1113,1	1440,4	1416,3	1456,4	1431,3
4	DRB1_1254_1r20	CATTGAAGAAWTAACACTapCaspA	10	1113,3	1440,4	1416,3	1456,4	1431,3
5	DRB1_1255_1r20	CRTTGAAGAAATGACACTspC^spA	+	1110,0	-1113,1	11.0,0		
		CATCTATAACCAACACCcnAAcnA	10	1162,1				1480,3
5 6		CATCTATAACCAAGAGGspA^spA CTTCTATCACCAAGARGspA^spG	10	1178,1	1505,4			1496,3
7		CTTCTATCACCAAGARCSpA spC	10	1178,1	1505,4			1496,3
8	DRB1_1963_1119	CGTCCATAACCAAGAGGspA^spG	10	1178,1	1505,4			1496,3
9		CATCTATAACCAAGAGGspA^spG	10	1178,1	1505,4			1496,3
10	DRB1_1965_1f19 DRB1_1966_1f19	CTTCCATAACCRGGAGGspA^spG	10	1178,1	1505,4		-	1496,3
11	DRB1_1966_1119 DRB1_1967_1f19	CTTCGATAACCAGGAGGspA^spG	10	1178,1	1505,4			1496,3
12	DRB1_1967_1119	CTTCTATAACCTGGAGGspA^spG	10	1178,1	1505,4		-	1496,3
13	DKB1_1900_1119		1					
0 14	DRB1_1971_1r20	CGTCGCTGTCGAAGCGCAspG^spG	10	1178,1	1505,4	-	-	1496,3
	DRB1_1971_1120	CGTCGCTGTCGTAGCGCGspC^spG	0	1154,1	-	-	-	1472,3
15 16	DRB1_1973_1r20	CGTCGCTGTCGAAGCGCAspA^spG	0	1162,1	-	-		1480,3
17	DRB1_1974_1r20	CGTCGCTGTCGAAGYGCAspC^spG	-28		1437,4	•	1453,4	1428,3
18	DRB1 1975_1r20	CGTCGCTGTCGAASCGCAspC^spG	-28	1110,1	1437,4	-	1453,4	1428,3
10	DKB1_1373_1720		1					
19	DRB1_2271_1f20	CGACAGCGACGTGGGGGAspC^spT	0	1113,1	1440,4		-	
20	DRB1_2272_1f20	CGACAGCGACGTGVGGGAspG^spT	0	1153,1	1480,4		-	1471,3
120	DIOI_LLI L_IIL							
15 21	DRB1_2611_1r20	TTCTGGCTGTTCCAGTACspT^spG	0	1231,2	-	-	1574,5	
22	DRB1_2612_1r20	TTCTGGCTGTTCCAGTACspC^spC	0	1074,1		1377,3		
23		TTCTGGCTGTTCCAGTAGspT^spC	0	1231,2		1534,4		
24	DRB1_2614_1r20	TTCTGGCTGTTCCAGTRCspT^spC	-14		1504,5	1480,4	1520,5	
25	DRB1_2615_1r20	TTCYGGCTGTTCCAGGACspT^spC	-14	1177,2	1504,5	1480,4	1520,5	
				<u> </u>	<u> </u>			
26	DRB1_2861_1f19	CTGGAACAGCCAGAAGAspA^spC	-28		1449,4		<u> </u>	4450.2
27	DRB1_2862_1f19	CTGGAACAGCCRGAAGGspA^spC	10	1138,1	1465,4	1441,3		1456,3
20				<u> </u>	<b> </b>	4404.0	<b> </b>	
28	DRB1_2991_1f20	GAAGGACHTCCTGGAGCAspG^spG	0	1178,1	<u> </u>	1481,3	4454.4	<u> </u>
29	DRB1_2992_1f20	GAAGGACATCCTGGGAGAspC^spA	-14		1435,1	<b> </b>	1451,4 1452,4	<u> </u>
30	DRB1_2993_1f20	GAAGGACATCCTGGARGAspC^spA	-14		1435,1	<del> </del>	1452,4	<u> </u>
31	DRB1_2994_1f20	GAAGGACYTCCTGGAAGAspC^spA	-14		1435,1		1505,4	<del>                                     </del>
32			10	1162,1	1489,4	<del></del>	1521,4	
33			10	1178,1	1465,4	<del> </del>	1021,4	
34	DRB1_2997_1f20	GAAGGACHTCCTGGAAGAspC^spG	0	1138,1	1400,4	<del></del>	<del></del>	<b></b>
[			-	1 400 4	<del> </del>	1441,3	-	<del>                                     </del>
25 35			10		1411 4	1387,3	<del></del>	1402,3
36			-14		1411,4 1411,4	1387,3		1402,3
37	DRB1_3083_1r20		-1-1-			1387,3		1402,3
38			-14		1411,4 1411,4	1387,3		1402,3
39		GTCTGCAGTAGGTGTCCAspC^spC	-14		1411,4	1387,3	1427,4	1402,3
40	DRB1_3086_1r20	GTCTGCAATAGGTGTCCAspC^spC	-14	1004,1	1 171 1,74	1 ,557,5	1	
	<u> </u>		<del>-   -</del>	14044	<del>                                     </del>	1497,3	<del>                                     </del>	1512,3
41	DRB1_341_1f19	TGCAGACACAACTACSGspG^spG	10	1194,1	<del> </del>	1731,0	<del>                                     </del>	† · · · · · · ·
30	<u> </u>	TO TOTAL TOTAL TALES	10	1191,3	1518,5	1494,4	<del>  .</del>	-
42		CGCTGCACTGTGAATCTCspT^spC			1518,5		-	1 .
43	DRB1_3452_1r20	CTCTGCACTGTGAAGCTCspT^spC	0		1518,5			<del>                                     </del>
44	DRB1_3453_1r20	CGCTGCACYGTGAAGCTCspT^spC		1191,3	.010,0	1 10 117	<u> </u>	<u> </u>

BNSDOCID: <WO\_\_\_\_2005052189A2\_I\_>

The resolution achievable by 19 markers each for HLA-A and HLA-B and the ten markers for HLA-DRB1 are listed in Tables VII to IX below.

**TABLE VII** 

Frequent Alleles of HLA-A	Group of frequent Alleles with same four- digit type	Rare Alleles with same Mini- Haplotype Profile	Resolution (in %)
A*0101	A*010101, A*010102	A*0103, A*0104N, A*0109	98,3
A*0201	A*02010101, A*020101021, A*020103, A*020104, A*020108, A*020109	A*0204, A*0209, A*0225, A*0231, A*0232N, A*0242, A*0243N, A*0253N, A*0258, A*0260, A*0264, A*0266, A*0267	93,4
	A*020102		100
	A*020105		100
	A*020106		100
	A*020107		100
A*0301	A*03010101, A*03010102N	A*0303N, A*0304, A*0305, A*0306, A*0311N	97,6
	A*030102		100
	A*030103		100
A*2301	A*2301	A*2306, A*2307N, A*2308N	98,6
A*2402	A*24020101, A*240201021, A*240202, A*240203, A*240204	A*2404, A*2409N, A*2411N, A*2426, A*2427, A*2432, A*2435, A*2436N, A*2437, A*2439	94,5
A*2902	A*290201	A*29010101; A*29010102N, A*2906, A*2908N	98,3
	A*290202		100
A*3001	A*3001		100
A*3002	A*3002		100

Capture: Alleles in a same field have the same mini-haplotype profile; grey high lighted are all alleles with identical sequences over exons 2 and 3.

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Frequent Alleles of HLA-B	Groups of frequent Alleles with same four-digit type	Rare Alleles with same Mini- Haplotype Profile	Resolution (in %)
B*0702	B*070201, B*070202, B*070203, B*070204	B*0703, B*0721, B*0722, B*0723, B*0730, B*0733, B*0735	98,0
B*0801	B*0801	B*0808N, B*0818, B*0819N	99,3
B*1302	B*1302	B*1308	99,6
B*1501	B*15010101; B*15010102N, B*150103, B*150104	B*1528, B*1533, B*1534, B*1560, B*1575, B*1578, B*1579N, B*1581, B*1582	97,6
	B*150102		100
B*1801	B*180101, B*180102	B*1805, B*1817N	99,3
B*3501	B*350101, B*350102	B*3507, B*3540N, B*3541, B*3542, B*5305	98,7
B*3503	B*3503	B*3536	99,6
B*4001	B*400101, B*400102,	B*4011, B*401401, B*401402, B*401403, B*4022N	98,7
	B*400103		100
	B*400104	B*4004	99,6
B*4402	B*44020101, B*44020102S, B*440202, B*440203	B*4411, B*4419N, B*4422, B*4423N, B*4427, B*4433, B*4434, B*4435	97,8
B*4403	B*440301	B*4413, B*4426, B*4429, B*4430, B*4432, B*4436, B*4437, B*4438, B*4439	98,2
	B*440302	B*4407	99,6
B*5101	B*510101, B*510102, B*510105	B*5111N, B*5112, B*5114, B*5118, B*5126, B*5127N, B*5128, B*5130, B*5132, B*5133	97,6
' !	B*510103		100
	B*510104	B*5124	99,6
B*5701	B*570101	B*5706, B*5708	99,5
	B*570102		100

Capture: Alleles in a same field have the same mini-haplotype profile; grey high lighted are all alleles with identical sequences over exons 2 and 3.

TABLE IX

Frequent Alleles of	Groups of frequent	Rare Alleles with same Mini-	Resolution
HLA-DRB1*	Alleles with same	Haplotype Profile	(in %)
	four-digit type		
DRB1*0101	DRB1*010101	DRB1*0105, DRB1*0107,	98,9
		DRB1*0111	
	DRB1*010102		100
DRB1*0301	DRB1*030101,	DRB1*0307, DRB1*0312,	97,2
	DRB1*030102	DRB1*0313, DRB1*0315,	-
	)	DRB1*0316, DRB1*0318,	
	<u></u> .	DRB1*0322, DRB1*0323	
DRB1*0401	DRB1*040101,	DRB1*0409, DRB1*0426,	98,6
	DRB1*040102	DRB1*0433	·
DRB1*0701	DRB1*070101,	DRB1*0703, DRB1*0704,	98,3
	DRB1*070102	DRB1*0705, DRB1*0707	, , ,
DRB1*1101	DRB1*110101,	DRB1*112701,	97,5
	DRBJ*110102,	DRB1*112702, DRB1*1130,	
1	DRB1*110103,	DRB1*1139	
1	DRB1*110104,		
	DRB1*110105		
DRB1*1104	DRB1*110401,	DRB1*1134, DRB1*1146	98,9
	DRB1*110402		
DRB1*1302	DRB1*130201,	DRB1*1331, DRB1*1339,	98,6
	DRB1*130202	DRB1*1341	ĺ
DRB1*1501	DRB1*150101,	DRB1*1503, DRB1*1506,	98,0
[	DRB1*150103,	DRB1*1509, DRB1*1513	,
	DRB1*150105		
	DRB1*150102		100
	DRB1*150104	DRB1*1512	99,4

Capture: Alleles in a same field have the same mini-haplotype profile; grey high lighted are all alleles with identical sequences over exon 2 (base 101 to 356)

The complete list of HLA alleles and sub-groups generated by the most informative mini-haplotyping markers (ten each for HLA-A, HLA-B and HLA-DRB1) are listed in Tables X to XII below.

																		7	Δ	B	L	F	X	,								•										
	Position		_	_	9	4	4	4	. 4	5 3	5 3	5	5	2	2 8	2	- 8	5	5 6	5	5 7	5	3	3	6	3	5	5	5 :	5   8	3 9	2 9	2	2		2	2		2' 7		2 2	
	CDNA A*3008	5 T				+	G	3 A				8 T				A	. G	7	8	_9	_0	1	5	6	7	A (	<u>6</u>	7_	89	9 9	0	_1	_ 2	8	9	0	1	2	0	_	2 :	3_
	A*6806	Т	С	T	A	. ⊤	G	A	A	A	G	т	G	c	С	A	Ģ	G	G	Α	G	T	т	G	Τ.	Α	Ç (	G	G /	4	٠, c	: Т	G	G	G	G	С	С	Ť	G	т (	3
	A*0244	Т	С	Т	A	c	С	. A	C	A	G	С	A	С	С	Α	Ċ	G	G	Α	G	Т	т	G	T ,	A	ii. Gil	G :	3 (	3 4	C	т	c	G	G	т	С	c	A	G	т (	3
5	A*0254	Т	С	7	Α	c	С	: A	C	A	G	С	Α	С	С	Α	C	G	G	Α	C	G	т	G	T	A	3	G	G (	3 4	\ C	т	C	Ğ	G	Т	С	С	Α	G	Т	3
	A*0205	T	С	Т	Α	С	С	A	Ċ	A	G	Т	G	С	С	Α	C	G	G	Α	G	T	Т	G	T	A	G (	G	3 (	3 4	, c	Т	С	G	G	Т	С	С	A	G	т	3
	A*0208	⊤	С	Т	Α	C	С	Α :	C	A	G	Т	G	С	С	Α	Ċ	G	G	Α	G	Ť	т	G	$T_j$	Α	G (	G	G	3 4	\ C	Τ	C	G	G	T	С	С	T	G	Т	3
	A*6815	T	С	T	Α	С	С	A	Ċ	A	G	Т	G	C	С	Α	Ċ	G	G	Α	G	Ţ	Т	G	T :	Α	Ġ,	G	3 /	A	C	Т	С	G	G	G	С	С	Τ.	G	т (	3
	A*6802 A*6818N	T	C	T	A	C	C	A	C	Â	G G	T T	GG	CC	C	A	G	G	G	A A	G G	T	T	G G	T T	A	o (	G G	G /		\ C	T	G	G	G G	G	C	C	Ť	G :	T C	3
	A*0228	T	С	T	Α	С	С	Α	C	A	G	Т	+	С	C	Α	C	G	G	Α	G	T	Т	G	Τ.	A	G, (	G (	3 (	Ā	C	Т	С	A	G	т	С	С	A	G .	T	3
10	A*0206 A*0214 A*0221 A*0257 A*0251	TTT	CCC	TTT	Α	CCC	0	A	C	A	9	T T	T	000	000	A	C	G	G	A	G∶ G∣		T	G	T T	AAAA	G (	G (	3 ( 3 (	S A	0	T	000	GGG	G	T T	000	000	A A	G :	T 6	3
	A*0261	Т	¢	τ	Α	С	С	Α	C	A	G	T	Ţ	С	С	Α	C	G	G	Α	G	Ţ	т	G	T	A	3 (	G (	3 (	3 A	C	Т	C	G	G	т	G	С	A	G ·	ТС	3
	A*0210	Т	С	7	Α:	С	С	A	C	A	G	T	T	С	С	Α	Ċ	G	G	Α	G	T	т	G	T	Ţ	Š (	G	3 (	∌ A	C	Т	C	G	G	T	С	С	Α	G ·	Τ, 6	3
	A*6901	Т	С	T	A	С	С	A	Ç	A	G	Т	Ţ	С	С	Α	G	G	G	Α	G	Τ	Т	G	T	A	9 (	G (	3 /	۸	C	T	G	G	G	G	С	С	T	G .	TG	3
15	A*2504 A*2608	T	С	T	Α	C	С	A	G	I۸	G	С	A	С	С	Α	Ç	G	G	Α	G	Т	Т	G	Т	A	3 (	G (	3 /	1	C	Т	G	G	G	G	C.	-C	T,	G .	TG	3
	A*2603 A*2606	T	C	T	A	C	C	A	G	A	G G	T	G	C	C	A	C	G	G G	A A	G G	T	T	G G	T (	A		G (	3 A	A	C	T ፕ	C	GO	G G	G G	C	C	ない	G .	Γ G	3
20	A*2610 A*2609 A*250101 A*250102 A*2601 A*2605 A*2611N A*2614 A*2617 A*2617 A*2604 A*6603 A*2612 A*2618	T T T T T T T T T T T T T T T T T T T	00000000000000	TTTTTTTTTTTT	A A A A A A A A A A A	0000000000000	0000000000000	A A A A A A A A A A	0000000000000	AAAAAAAAAA	0000000000000	T T T T T T T T T T T T T T T T T T T	000000000000000000	00000000000000	00000000000000	A A A A A A A A A A	90000000000000	9999999999999	0000000000000	<b>AAAAAAAAA</b>	000000000000000	TTTTTTTTTT	***************************************	6666666666666	TTTTTTTTTTTTT	A A A A A A A A A A A			6	A A A A A A A A A A A A	0000000000000	***************************************	000000000000000	00000000000000	00000000000000	0000000000000	0000000000000	0000000000000		666666666666666666666666666666666666666		
	A*4301	Т	С	T	A	С	С	Α	G	Α	G	Т	G	С	С	Α	C	G	G	Α	G	T	Т	G	T !	۸		Т (	3 (	A	С	Т	G	G	G	G	С	С	T	G T	re	3
-	A*260701	Ì .	Ü	•	7	С	С	Α	G	Α	G	T.	G				C	.[		Α	į	T		G	- 3		8			A			G			G			A	G 1	r e	;
<b>~</b>	A*260702	Т	С	.T	A	С	С	Α	G	Α	G	T	G	С	С	Α	Ç	G	G	Α	G	Ţ	T	G	Τ.	A	3 (	3 (	3 6	A	C	т	G	G	G	٢	С	С	A'	G 7	ī G	;
25	A*2619	Т	С	Т	A	С	С	Α	G	Α	G	T	G	С	С	Α	G	G	G	Α	G	Т	T	G	T	Ä	)   	4 (	3 6	A	C	Т	G	A	G	G	С	c	Ť.	G 1	ī G	į
	A*3401 A*3405	Т	С	Т	A	С	С	Α	G	Α	G	Т	G	С	С	A	G	G	G	Α	G	Ţ	T	G	T .	A C	3 (	3 (	3 A	A	C	T	G	G	G	G	С	c /	A.	G 7	ΓG	3
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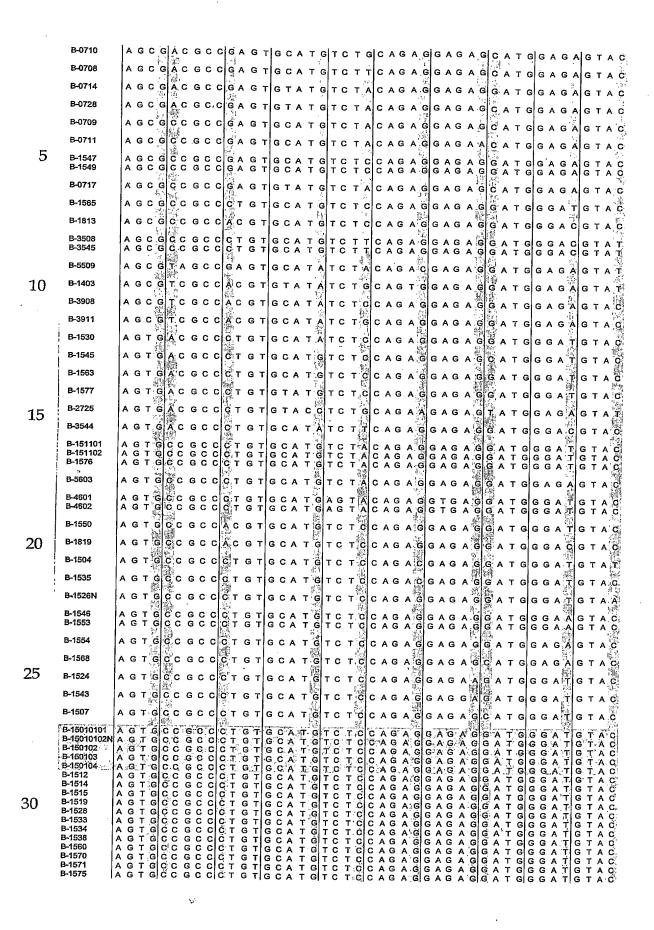
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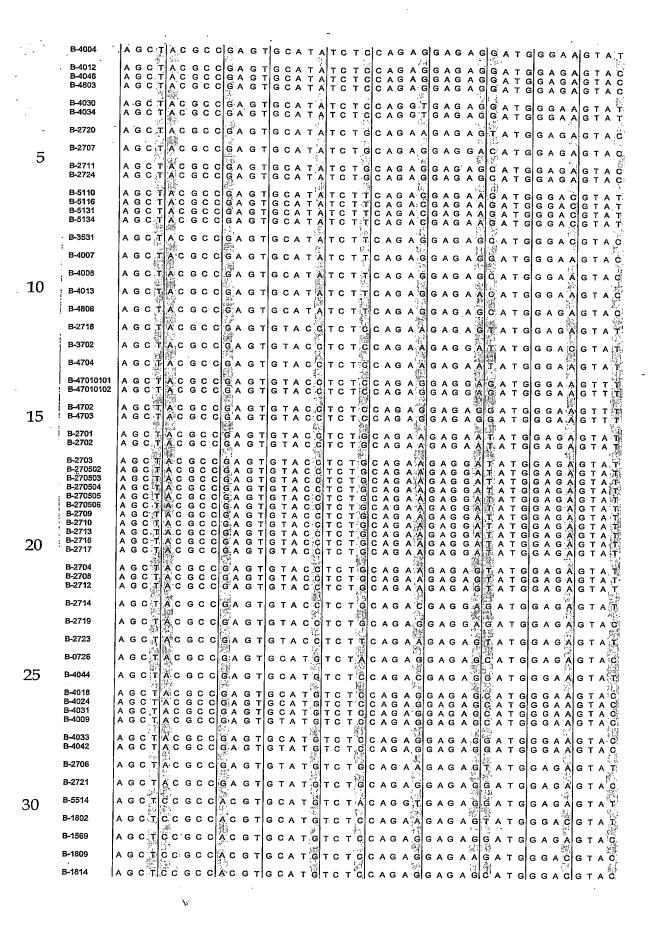
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	B-4805	A G C G A C G C C G A G T G C A T A T C T C C A G A G G A G A G G A T G G A G A G T	A C
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25	B-070201 B-070203 B-070204 B-0703 B-0716 B-0721 B-0722 B-0723 B-0723 B-0733 B-0733	A G C G A C G C C G A G T G C A T G T C T A C A G A G G A G A C G A T G G A G A G T A G C G A C G C A T G G A G A G T C T A C A G A G G A G A G C A T G G A G A G T A G C G A C G C C C G A G T G C A T G T C T A C A G A G G A G A G C A T G G A G A G T A G C G A C C G C C G A G T G C A T G T C T A C A G A G G A G A G C A T G G A G A G T A G C G A C C G C C G A G T G C A T G T C T A C A G A G G A G A G C A T G G A G A G T A G C G A C G C C G A G T G C A T G T C T A C A G A G G A G A G C A T G G A G A G T A G C G A C G C C G A G T G C A T G T C T A C A G A G G A G A G C A T G G A G A G T A C G C C G A G T G C A T G T C T A C A G A G G A G A G C A T G G A G A G T A C C G A C C C C G A G T G C A T G T C T A C A G A G G A G A G C A T G G A G A G T C T A C A G A G G A G A G C A T G G A G A G T C T A C A G A G G A G A G C A T G G A G A G C A T G T C T A C A G A G G A G A G C A T G G A G A G C A T G T C T A C A G A G G A G A G C A T G G A G A G C A T G T C T A C A G A G G A G A G C A T G G A G A G C A T G T C T A C A G A G G A G A G C A T G G A G A G C A T G T C T A C A G A G G A G A G C A T G T C T A C A G A G G A G A G C A T G G A G A G C A T G T C T A C A G A G G A G A G C A T G T C T A C A G A G G A G C A T G T C T A C A G A G G A G A G C A T G T C T A C A G A G G A G A G C A T G T C T A C A G A G G A G A G C A T G T C T A C A G A G G A G C A T G T C T A C A G A G G A G C A T G T C T A C A T G T C T A C A T G T C T A C A T G T C T A C A T G T C T A C A T G T C T A C A T G T C T A C A T G T C T A C A T G T C T A C A T G T C T A C A T G T C T A C A T G T C T A C A T G T C T A C A T G T C T A C A T G T C T A C A T G T C T A C T C T A C T C T A C A T G T C T A C T C T A C T C T A C T C T A C T C T	00000000000000000000000000000000000000
	B-0707	A G C G A C C C G A G T G C A T G T C T A C A G A G G A G G A T G G A G A G T	15
	B-0712 B-0718	A G C G A C C C G A G T G C A T G T C T A C A G A G G A G G A T G G A G A G G A T G G A G A	
00	B-0736	A G C G A C C G A G T G C A T G T C T A C A G A G G A G A T G G A G A G T	O.
30	B-0715	A G C G A C C C G A G T G C A T G T C T A C A G A G G T G A G C A T G G A G A G T	17
	В-0727	A G C G A C G C C G A G T G C A T G T C T C C A G A G G A G A A T G G A G A G T	A C
	B-4032	A G C G A C C G A G T G C A T G T C T C C A G A G G A G C A T G G G A A G T	A C
	B-4808	A G C G A C G C C G A G T G C A T G T C T C C A G A G G A G G A T G G A G A G T	A C



	B-1578 B-1579N B-1581 B-1582	A G T G C C G C C T G T G C A T G T C T C C A G A G G A G A G G A T G G G A T G A G T G C C G C C C T G T G C A T G T C T C C A G A G G A G A G G A T G G G A T G A G T G C C C C T G T G C A T G T C T C C A G A G G A G A G G A T G G G A T G A G T G C C C C T G T G C A T G T C T C C A G A G G A G A G G A T G G G A T G A G T G C C C C T G T G C A T G T C T C C A G A G G A G A G G A T G G G A T G T C T C C A G A G G A G A G G A T G G G A T G T C T C C A G A G G A G G A T G G	TAC.
	B-1527	A G T G C C C C T G T G C A T G T C T C C A G A G G A G G A T G G G A T G	7.2
	B-1532	A G T G C C C C T G T G C A T G T C T C C A G A G G A G G A T G G G A T G	4
	B-1557	A G T G C C G C C C T G T G C A T G T C T C C A G A G G T G A A G A T G G G A T G	TAC
5	B-1566	A G T G C C C C T G T G C A T G T C T G C A G A G G A G G A T G G G A T G	TAC
5	B-1508 B-1556	A G T G C C C C T G T G C A T G T C T T C A G A G G A G A T G G G A T G A G T G G A T G G A T G G G A T G G G A T G G G A T G G G A T G G G A T G G G A T G G A T G G A T G G A T G G A T G G A T G G A T G G	T A C
	B-351401 B-351402	A G T G C C G C C T G T G C A T G T C T T C A G A G G A G A G G A T G G G A C G A G G C G C C T G T G C A T G T C T T C A G A G G A G G A T G G G A C G	
	B-3543	A G T G C C C C T G T G C A T G T C T T C A G A G G A G G A T G G G A C G	TAC
	B-1573	A G T G T A G C C C T G T G C A T A T C T C C A G A G G A G G A T G G G A T G	TAC
	B-1558	A G T G T C G C C C T G T G C A T A T C T C C A G A G G A G G A T G G G A T G	TAC
10	B-3918	A G T GTC G C CAC G T G C A T A T C T G C A G A G G A G G A T G G A G A G	TAC
10	B-0734 B-5504	A G C T A C G C C A C G T G C A T A T C T A C A G A G G A G A G G A T G G A G A G A G	TAC TAC
i	B-5612	A G C TAC G C CAC G T G C A TAT C TAC A G A G G A G A G G A T G G A G A G	TAC
	B-4039	A G C TAC G C CAC G T G C A TAT C T G C A G A G G A G A G G A T G G G A A G	TAC
	B-2715	A G CTAC G C CAC G T G T A C G T C T G C A G A A G A G A G A T G G A G A G	TAT
	B-3914	A G C T A C G C C A C G T G C A T A T C T G C A G A G G A G A G C A T G G A G A G	TAO
I I	B-0813	A G CTTAC G C C A C G T G C A T A T C T T C A G A G G A G A G C A T G G A G A G	TAC
15	B-5121	A G C T AC G C C A C G T G C A T A T C T T C A G A G G A G A A G A T G G G A G	TAT
	B-5508	A G CATAC G C C CT G T G C A TA T C TA C A G A G G A G A G A G A G A G A G A	門袋
	B-560501 B-560502	A G CTHÁC G C C OT G T G C A TÁIT C TÁIC A G A C G A G A G G A T G G A G Á G A G C TÁC G C C G T G T G C A TÁIT C TÁIC A G A C G A G A G G A T G G A G A G	
	B-5606	A G C TAC G C C T G T G C A T A T C T A C A G A C G A G A T G G G A C G	TAT
,	B-1548	A G C TAC G C C T G T G C A TAT C T C C A G A G G A G A G G A T G G G A T G	TAC
	B-4005	A G C TIAC G C C C T G T G C A T A T C T C C A G A G G A G A G G A T G G G A A G	
	B-4026	A G C T C C G C C T G T G C A T A T C T C C A G A G G A G A G G A T G G G A T A G	9/6/4
20	B-4028	A G C TIAC G C C C T G T G C A T A T C T C C A G A G G A G A G G A T G G G A A G	$\frac{i_2^{-1}}{i_1 A_1} \frac{1}{A}$
	B-5107 B-520101 B-520102 B-520103 B-520104 B-5203 B-5204 B-5205	A G C T A C G C C G T G T G C A T A T C T G C A G A C G A G A A G A T G G G A C G A G C T A C G C C G T G T G C A T A T C T C C A G A C G A G A A G A T G G G A C G A G C T A C G C C C T G T G C A T A T C T C C A G A C G A G A A G A T G G G A C G A G C T A C G C C C T G T G C A T A T C T C C A G A C G A G A A G A T G G G A C G A G C T A C G C C C T G T G C A T A T C T C C A G A C G A G A A G A T G G G A C G A G C T A C G C C C T G T G C A T A T C T C C A G A C G A G A A G A T G G G A C G A G A T G G G A C G A G A T G G C T A C G C C C T G T G C A T A T C T C C A G A C G A G A A G A T G G G A C G A G C T A C G C C C T G T G C A T A T C T C C A G A C G A G A A G A T G G G A C G A G C T A C G C C C T G T G C A T A T C T C C A G A C G A G A A G A T G G G A C G A G C T A C G C C C T G T G C A T A T C T C C A G A C G A G A A G A T G G G A C G A G C T A C G C C C T G T G C A T A T C T C C A G A C G A G A A G A T G G G A C G A G C T A C C G C C C T G T G C A T A T C T C C A G A C G A G A A G A T G G G A C G A G C T A C C G C C C T G T G C A T A T C T C C A G A C G A G A A G A T G G G A C G A G C T A C C G C C C T G T G C A T A T C T C C A G A C G A G A A G A T G G G A C G A C G A G A C G A	T A T T A T T A T T A T
	B-5202	A G C TAC G C C G T G T G C A T A T C T C C A G A C G A G A A G A T G G G A T G	TAT
	B-7805	A G C A C G C C C T G T G C A T A T C T C C A G A C G A G A G G A T G G G A C G	TAT
25	B-1509	A G C TAC G C C C T G T G C A TAT C T G C A G A G G A G A G G A T G G A G A G	TAC
	B-5122	A G C TAC G C C T G T G C A TAT C T G C A G A G G A G A A G A T G G G A C G	TAT
	B-7803	A G C TAC G C C C T G T G C A TAT C T G C A G A C G A G G A T G G G A C G	TAT
	B-570301 B-570302 B-5707	A G C T A C G C C T G T G C A T A A C A T C A G G T G A G A A G A T G G G A T G A G C T A C G C C T G T G C A T A A C A T C A G G T G A G A A G A T G G G A T G A G C T A C G C C T G T G C A T A A C A T C A G G T G A G A A G A T G G G A T G	TAT
	B-5808	A G C TA C G C C C T G T G C A T A A C A T C A G A C G A G A A G A T G G G A C G	TAT
30	B-510101 B-510102 B-510103 B-510104 B-510105 B-510201 B-510202 B-5103 B-5109	A G C T A C G C C C T G T G C A T A T C T T C A G A C G A G A A G A T G G G A C G A G C T A C G C C T G T G C A T A T C T T C A G A C G A G A A G A T G G G A C G A G C T A C G C C T G T G C A T A T C T T C A G A C G A G A A G A T G G G A C G A G A A G A T G G G A C G A G A A G A T G G G A C G A G A A G A T G G G A C G A G A A G A T G G G A C G A G A A G A T G G G A C G A G A A G A T G G G A C G A G A A G A T G G G A C G A G A A G A T G G G A C G A G A A G A T G G G A C G A G A A G A T G G G A C G A G A A G A T G G G A C G A G A A G A T G G G A C G A G A A G A T G G G A C G A G A A G A T G G G A C G A G A G A A G A T G G G A C G A G A G A G A T G G G A C G A G A G A G A T G G G A C G A G A G A G A T G G G C C C C T G T G T G T T T T T T	T A T T A T T A T T A T
	B-5111N B-5112	A G C TAC G C C C T G T G C A TAT C T T C A G A C G A G A A G A T G G G A C G A G C T A C G C C C T G T G C A T A T C T T C A G A C G A G A A G A T G G G A C G	TAT

. 5	B-5114 B-5118 B-5119 B-5123 B-5126 B-5127N B-6128 B-5130 B-5130 B-5133 B-7801 B-780201 B-780202 B-7804 B-350901 B-350901	A A A A A A A A A A A A A A A A A A A	CCCCCCCCC CCCC CCCCCCCCCCCCCCCCCCCCCCC	00000000000000000000000000000000000000	GCCATTGGCATTTGGCATTTGGCAATTTGGCAATTTGGCAATTTGGCAATTTGGCAATTTGGCAATTTGGCAATTTGGCAATTTGGCAATTTGGCAATTTTGGCAATTTTGGCAATTTTGGCAATTTTTGGCAATTTTTTTT	AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	GGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	G G G G A C C G G T A T T G G G G A C C G G T A T T A T T G G G G A C C G G T A T T G G G G A C C G G G A C C G G G A C C G G G A C C G G G A C C G G G A C C G G G A C C G G G A C C G G G A C C G G G G
10	B-3522 B-5104 B-5106 B-5117 B-15170101 B-15170102	A G C		C C C C C C C C C C C C C C C C C C C	T G C A T Z T G C A T Z T T T A T Z	T C T		BAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG	A T G G G A C G T A C A T G G G A C G T A C A T G G G A C G T A T A T G G G A C G T A T A T G G G A C G T A T A T G G G A C G T A T A T G G G A T G T A C
15	B-1510 B-1537 B-3534 B-3539 B-440301 B-4413 B-4429 B-4429 B-4430 B-4432 B-4436 B-4437 B-4438 B-4438 B-4439	CC C C CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC			T G C A T G C A T G G C A T G G C A T G G T A	T C T C T C C T C C C T C C C T C C C T C C C T C C C T C	AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	G G G G G G G G G G G G G G G G G G G	A T G G G A A A G G T A A C G G G A A A G G T A A C G G A A A G G T A A C G G G A A A G G T A A C G G G A A A G G T A A C G G G A A A G G T A A C G G G A A A G G T A A C G G G A A A G G T A A C G G G G A A A G G T A A C G G G G A A A G G T A A C G G G G G A A A G G T A A C G G G G G A A A G G T A A T G G G G G G
20	B-440302 B-4407 B-8101 B-8102 B-40060101 B-40060102 B-4019 B-4037 B-4047	A G G C C C C C C C C C C C C C C C C C		CC CC CC CC CC CC CC CC CC CC CC CC CC	GTATGGTATGGCATA	T G T G T C T A T C T	C A G G G G G G G G G G G G G G G G G G	A G A A A G A A A G A A A G A A A G A A A G A A A G A A A G A A A A G A A A G A A A G A A A A G A	A T G G G A A G T A C A T G G G A A G T A T A T G G G A A G T A T A T G G A G A G T A C A T G G A G A G T A C A T G G G A A G T A T A T G G G A A G T A T A T G G G A A G T A T A T G G G A A G T A T A T G G G A A G T A T
25	B-4431 B-4002 B-4027 B-4029 B-4035 B-4040 B-4045	A G G G G G C C C C C C C C C C C C C C		A G T A G T	G C A T A G C A T A G C A T A G C A T A G C A T A	T C T C T C T C T C T C T C T C T C T C	C A G A G G G C C A G A G G G C C A G A G	A A A A A A A A A A A A A A A A A A A	A T G G G A A G T A C C A T G G G A A G T A C C A T G G G A A G T A C C A T G G G A A G T A C C A T G G G A A G T A C C A T G G G A A G T A C C A T G G G A A G T A C C A T G G G A A G T A C C A T G G G A A G T A C C A T G G G A A G T A C C A T G G G A A G T A C C A T G G G A A G T A C C A T G G G A A G T A C C A T G G G A A G T A C C A T G G A G T A C C A T G G A G T A C C A T G G A G T A C C A T G G A G T A C C A T G G A A G T A C C A T G G A G T A C C A T G G A A G T A C C A T G G A A G T A C C A T G G A A G T A C C A T G G G A A G T A C C A T G G G A A G T A C C A T G G G A A G T A C C A T G G G A A G T A C C A T G G G A A G T A C C A T G G G A A G T A C C A T G G G A A G T A C C A T G G G A A G T A C C A T G G G A A G T A C C A T G G G A A G G T A C C A T G G G A A G G T A C C A T G G G A A G G T A C C A T G G G A A G G T A C C A T G G G A A G G T A C C A T G G G A A G G T A C C A T G G G A A G G T A C C A T G G G A A G G T A C C A T G G G A A G G T A C C A T G G G A A G G T A C C A T G G G A A G G T A C C A T G G G A G G T A C C A T G G G A G G T A C C C A T G G G A G G T A C C C A T G G G A G G T A C C C A T G G G A G G T A C C C A T G G G A G G T A C C C A T G G G A G G T A C C C A T G G G A G G T A C C C A T G G G A G G T A C C C A T G G G A G G T A C C C A T G G G A C G T A C C C A T G C C A T G C C A T G C C A T G C C A T G C C A T G C C A T G C C A T G C C A T G C C A T G C C A T G C C A T G C C A T G C C A T G C C A T G C C A T G C C A T G C C A T G C C C A T G C C A T G C C C A T G C C C A T G C C C A T G C C C A T G C C C A T
30	B-400101 B-400102 B-400103 B-4010 B-4011 B-401401 B-401402 B-401403 B-4022N B-4025 B-4043 B-4021	AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA		G A G T T G A A G T T G A A G T T G A A G T T G A A G T T G A A G T T G A A G T T G A A G T T T G A A G T T T G A A G T T T G A A G T T T T	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	C C C C C C C C C C C C C C C C C C C	C A G A G G G G C A G G A G G G G C A G G A G G G G		A T G G A A G T A C A T G G G A A G T A C A T G G G A A G T A C A T G G G A A G T A C A T G G G A A G T A C A T G G G A A G T A C A T G G G A A G T A C A T G G G A A G T A C A T G G G A A G T A C A T G G G A A G T A C A T G G G A A G T A C A T G G G A A G T A C A T G G G A A G T A C A T G G G A A G T A C A T G G G A A G T A C A T G G G A A G T A C A T G G G A A G T A C A T G G G A A G T A C



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	B-180101 B-180102	HI AUG COT	CC G C C A	CGTGCAT	GIT C T (	CAGAG	CAGAGG	ATGGGGACGTAC
	B-1803 B-1804	A G C T	CCGCCA	CGTGCAT	GILLI	JU AG AG	G A G A G G	A T GIG G A CICTAC
	B-1805 B-1811	AGCT	CCGCCA	CGTGCAT	G ТС Т (	C A G A G	GAGAGG	A T G G G A C G T A C A T G G G A C G T A C
	B-1812 B-1815 B-1817N	AGCT	C C G C C A	CGTGCAT	G ТС Т (	CAGAG	G A G A G G	ATGGGACGTAC
E	B-1808	#.u	C C G C C A		G T C T C	-1	2 199	ATGGGACGTAC
5	B-1818	AGCT	CCGCCA	CGTGCAT	:1	i (i. l	427 1.3	
	B-1806	22.	(48) h = 1	:	G тс т с	1 4.3	1 1	ATGGGACGTAC
	, B-3907	AGCT	C G C C A	CGTGCAT	G тс т		107 12	ATGGAGAGTAC
	B-1544	AGCT	CCGCCA	CGTGTAT	Стст	CAGAG	GAGAGG	ATGGGATGTAT
	B-1807	AGCT	CCGCCA	CGTGCAT	в тст т	CAGAG	GAGAGG	ATGGGACGTAC
	B-3535	AGCT	CCGCCA	CGTGCAT	з тстт	CAGAG	GAGAGG	ATGGGACGTAT
10	B-5805	AGCT	CCGCCA	CGTGCAT	A C A T	CAGAG	GAGAAG	ATGGGACGTAT
10	B-5609	AGCT	CCGCC	TGTGCAT	втс та	CAGAG	GAGAGG	ATGGAGAGTAT
	B-1304	AGCT	CCGCCC	TGTGCAT	этс т о	CAGAC	GAGAAG	ATGGGATGTAT
	B-5309	AGCT	ececce	TGTGCAT	зтс т с	CAGAG	GAGAAG	ATGGGACGTAT
	B-1503 B-1561	AGCT	CCGCCC	T G T G C A T C	TCTC	CAGAG	GAGAGG	A T G G A G A G T A C A T G G A G A G T A C
	B-1564 B-1574		C C G C C C	T G T G C A T C	SIIC TC	CAGAG	GAGAGG	ATGGAGAGTAC ATGGAGAGTAC
	B-1505 B-1539			T G T G C A T C	зтс т с	CAGAG	GAGAGG	A T G G G A T G T A C A T G G G A T G T A C
15	B-1562		CCGCCC		от с т с	CAGAG	GAGAGG	ATGGAGAGTAT
	B-4802 B-1520		源 上海	GIGCAT	TCTC	CAGAG	GAGAGG	ATGGAGAGTAT
	B-3520		CCGCCC	i .	4	3.00	部 45	ATGGGATGTAT
	B-3528	AGCT	C C C C C	TGTGCAT	TC T C	CAGAG	G A G A G G G A G A G G	ATGGGACGTAT ATGGGACGTAT
	B-1531	AGCT	CCGCC	TGTGTATG	тстс	CAGAG	GAGAGG	ATGGGATGTAC
	B-1555	433	CCGCCC	TGTGTAT	1 1	1 20	######################################	ATGGGATGTAC
20	B-1513 B-1536		C G C C C	T G T G T A T G	T C T C	CAGAG CAGAG	GAGAAG GAGAAG	ATGGGATGTAT ATGGGATGTAT
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15	DRB1-1344	C	Τ (	3 A	G	Α (	3.1	A	С	GΤ	A	G	T	T	O	G.	Α (	3	3 A	c	c	<b>A</b> (	3 <i>F</i>	١G	C	G <b>G</b>	T	G	G	3 1	Т	G	ΤC	The state of
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30 .	DRB1-1126	C	TO	Α	G	A G	T	A	C	GΤ	Α	G	T	T	G (	G /	۹ 0	3 0	A 6	C	2/	<b>4</b> G	A é	G	С	GG	Т	G	GG	<b>;</b>	Т	G (	ЭŢ	ļ
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. 5	DRB1-1125	Ç.	TG	3 A	G	A G	T	A	C	ЭT	Α	G	T	T	G	Α	G	G A	4 C	I	A (	C /	٩G	T	GG	Т	G	G	3 Т	Т	G.	ΤG
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15	DRB1-131402	C	TC	3 A	G	A G	; T	A	C,	3 T	A	G	<b>工</b>	T	r G	A	G	G /	A C	T	Α	C /	٩G	С	GG	T	G	G	3	Т	G	GT
10	DRB1-0304	С	ТС	ЭА	G	ΑG	() () ()	C	C	ЭТ	A	G	T	TC	C G	A	G	G /	A C	C	Α	G /	ΑĀ	G	GG	T	G	G	3 .T	┰	G	ΤG
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	DRB1-1360	C	TO	3 A	G	A G	計劃	C	C	3 T	A	G	Т	A	r G	Α	G	G /	A C	C	A	C /	٩G	C	GG	Т	G	G	3 T	Т	G	GТ
20	DRB1-1441	C B	Τ (	3 A	G	A G	T.	T	C	СΤ	A	G	T	A	) (	A	G	G /	A C	C	A	G /	A G	С	GG	۲	G	G	3 T	Т	G	GT
20	DRB1-1308 DRB1-1319	C	T (	3 A	G	A G	T	1	C	3 T	A	G	T	A	0	A	G	G /	A C	A	A	C	ЗΑ	Ç	GG	T	G	G	3 ∏ 3 ∵	Ţ	G	T G T G
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<b>0</b> E	DRB1-1357	C	TC	3 A	G	A G	T Isla	T	C	ЭТ	Α	G	T	T	G	A	G	G,	A C	Α	Α	C	GΑ	С	GG	Т	G	G	э 🏋	Т	G	ΤĞ
25	DRB1-0321	С	T	3 A	G	A G	Ţ		C	ЭТ	A	G	T	T	C	Α	G	G A	A C	C	Α	G /	AΑ	G	GG	Т	G	G	Э Т	T	G	TG
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	DRB1-1117	C	T C	3 A	G	A G	; T	門工	C	3 T	A	G	T	A	3 G	A	G	G A	A C	C	G	G /	A C	A	GG	Ţ	G	G	3 T	T	G '	ΤĠ
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30	DRB1-1438 DRB1-1439	C	TC	ЭΑ	G	A G	Ť	T	C (	ЭT	A	G	T	A	3 C	A	G	G,	A C	C	G	G,	A C	A	GG	Т	G	G	GT	T	G	T G T G
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	DRB1-1437	СТ	G	AG	A &	G ·	Γ	С	GT	1	A G	Т	A	T (	3 A	۱ G	G	Α(	C A	Α	G	3 C	c	GG	Т	G	GG	Т	T	G 1	G
	DRB1-1445	СТ	G.	AG	A é	G ·	г	С	GΤ		۹ G	Т	Α	Т	3 <i>P</i>	١G	G	A (	C A	G	G,	4 G	A	GG	Т	G	GG	Т	T	G 7	G
5	DRB1-140501 DRB1-1443																							GG GG							
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	DRB1-1440	СТ	G	A	A E	G	T	С	GΤ	- /	A G	T	Α	C	G A	A G	G	Α	C C	A	C.	A C	T	GG	T	G	ĢG	Т	T	G (	3 T
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	DRB1-0818 DRB1-0825	G T	G	AC	A 6	G	T T	C	G1 G1	-   ,	4 G	3 T	A	CC	G A	A G	G	A A	C A	A	C	A C	C	GG	T	G G	G G	T	T ·	G (	∋ T ∋ T
	DRB1-0810	G T	G	A	3 A	G;	T	C	G٦	- /	٩G	T	Α	C	G A	۹ G	G	Α	C A	A	С	A C	T	GG	Τ	G	GG	T	Τ,	G T	r G
05	DRB1-0812	G T	G	A	3 A	G	TA	C	G٦	- /	4 G	T	A	Ċ	G A	۹ G	G	Α	C A	A	С	A C	Ŧ	GG	T	G	GG	С	T	G 7	r G
25	DRB1-080302 DRB1-0814 DRB1-0819 DRB1-0823	G T	G	A G	3 A 3 A	G	T A	C	G1		4 G 4 G	T	A	CC	G A G A	4 G 4 G	G	A A	C A	A	C C	A (	JT JT	66 66 66	T	G G	G G G G	T T	T	G (	3 T 3 T
	DRB1-0813	G T	G	A	3 A	G	T	C	G1	-   ,	4 G	T	Α	С	G A	A G	G	Α	C C	A	С	A C	1	GG	Т	G	GG	Т	Т	G (	3 T
30	DRB1-080401 DRB1-080404 DRB1-0806	G T	G	A	3 A	G	T A	C	G1	-   /	٩G	T	Α	C	G A	A G	G	Α	C T	ĺΑ	С	A C	ЯT	GG GG	T	G	GG	T	T	G T	ΓG
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5	DRB1-080402 DRB1-080403	GG	T G	A A	G G	A G A G	T	A	C	GT GT	A	G	T T	A	Ç (	G ,	A C	G G	; А ; А	C T	T A	C	A C	でして	G C	€ T	G G	GG GG	Ţ	T	G .	т т. т т
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	DRB1-0815	G	ΤG	A	G	A G	Ţ	A	C	GΤ	Α	G	T	Α	G	G ,	A G	G	A	C/	A	С	ΑÇ	T	GG	F T	G	G G	∰**. <b>T</b>	Т	G	3 <b>T</b>
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General strategy for medium resolution typing is described below:

For medium resolution typing a maximally informative set of marker positions were determined. These consist of positions 98, 414, 539, 282, 571, 368, 256, 292, 238, 270, 453, 527, 502, 81, 268, 559, 92, 123 and 396 of HLA-A (numbering starts at the transcription start position of exon 1), positions 539, 419, 559, 412, 272, 362, 302, 363, 206, 369, 259, 97, 583, 292, 222, 527, 418, 435 and 571 of HLA-B (numbering starts at the transcription start position of exon 1), and positions 125, 196, 197, 227, 261, 286, 299, 308, 341 and 345 of HLA-DRB1 (numbering starts at the transcription start position of exon 1).

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In general, the order of the positions is from the most informative to the least informative with respect to the selection criteria of frequent and rare HLA alleles (see list of frequent HLA alleles above). Thus the ten markers (HLA-A and HLA-B) that were selected for the fine typing strategy constitute the first ten markers of the set of 19 markers for the single pass classification into frequent and rare HLA alleles (HLA-A and HLA-B). Like with sequence-based HLA typing there are heterozygous combinations of HLA alleles that can not be resolved. However, there are fewer ambiguities with this method due to the mini-haplotypes that are provided.

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Another object of the present invention is the use of said methodology of the invention is for screening of tissue donors, for example, bone marrow donors in registries for frequent and rare HLA types.

The description of the HLA alleles is based on the Anthony Nolan database (www.ebi.ac.uk/imgt/hla/).

In addition to the aforementioned method, the invention includes yet other arrangements which will emerge from the description that follows, which refers to examples of supports according to the invention, as well as the annexed figures and tables, wherein:

WO 2005/052189 PCT/IB2004/004115

Figure 1 describes 19 positions covered by mini-haplotyping assays for discrimination of HLA-A mapped onto the HLA-A allele A\*010101 as reference. Black boxes indicate an extension position while grey boxes indicate polymorphisms that are captured by the annealing of the respective primer of the primer pool. Pools are used in forward and reverse. Numbering is according to the transcription start of the cDNA.

Figure 2 describes 19 positions covered by mini-haplotyping assays for discrimination of HLA-B mapped onto the HLA-B allele B\*070201 as reference. Black boxes indicate an extension position while grey boxes indicate polymorphisms that are captured by the annealing of the respective primer of the primer pool. Pools are used in forward and reverse. Numbering is according to the transcription start of the cDNA.

Figure 3 describes 10 positions covered by mini-haplotyping assays for discrimination of HLA-DRB1 mapped onto the HLA-DRB1 allele DRB1\*0101 as reference. Black boxes indicate an extension position while grey boxes indicate polymorphisms that are captured by the annealing of the respective primer of the primer pool. Pools are used in forward and reverse. Numbering is according to the transcription start of the cDNA.

Figure 4 describes 10 positions covered by mini-haplotyping assays for discrimination of HLA-A mapped onto the HLA-A allele A\*010101 as reference for the distinction of subgroups that can then be further analysed. Black boxes indicate an extension position while grey boxes indicate polymorphisms that are captured by the annealing of the respective primer of the primer pool. Pools are used in forward and reverse. Numbering is according to the transcription start of the cDNA.

Figure 5 describes 10 positions covered by mini-haplotyping assays for discrimination of HLA-B mapped onto the HLA-B allele B\*070201 as reference for the distinction of subgroups that can then be further analysed. Black boxes indicate

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an extension position while grey boxes indicate polymorphisms that are captured by the annealing of the respective primer of the primer pool. Pools are used in forward and reverse. Numbering is according to the transcription start of the cDNA.

Figure 6 shows genotyping results of a CEPH family (1418, 01 = father, 02 = mother, 03 = child, 04 = child) for position HLA-B\_272. 1407,3 Da corresponds to the addition of C to primer 6, 7, 8, or 9; 1422,3 Da corresponds to the addition of T to primer 6, 7, 8, or 9; 1431,4 Da/ 1430,9 Da corresponds to the addition of A to primer 6, 7, 8, or 9; and 1447,4 Da/ 1448,5 Da corresponds to the addition of G to primer 6, 7, 8, or 9.

Table I represents HLA-A alleles captured by the 10 markers in the different subgroups and additional positions that have to be typed to resolve the subgroups.

Table II represents HLA-B alleles captured by the 10 markers in the different subgroups and additional positions that have to be typed to resolve the subgroups.

Table III represents HLA-DRB1 alleles captured by the 10 markers in the different subgroups and additional positions that have to be typed to resolve the subgroups.

Table IV represents the list of the individual primers that are required to constitute the pools for mini-haplotyping of HLA-A (19 markers). The 10 markers required for the creation of subgroups are also contained. ^ refers to the base used to attach the mass/charge tag, CT refers to the mass difference of the mass/charge tag, sp

means phosphorothioate group. The product analysed by mass spectrometry includes the base 5' of the most 5' phosphorothioate (sp).

Table V represents the list of the individual primers that are required to constitute the pools for mini-haplotyping of HLA-B (19 markers). The 10 markers required for the creation of subgroups are also contained. ^ refers to the base used to attach the mass/charge tag, CT refers to the mass difference of the mass/charge tag, sp

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means phosphorothicate group. The product analysed by mass spectrometry includes the base 5' of the most 5' sp.

Table VI represents the list of the individual primers that are required to constitute the pools for mini-haplotyping of HLA-DRB1 (10 markers). ^ refers to the base used to attach the mass/charge tag, CT refers to the mass difference of the mass/charge tag, sp means phosphorothioate group. The product analysed by mass spectrometry includes the base 5' of the most 5' sp.

Table VII represents the resolution that can be generated with the 19 markers for the distinction of the frequent HLA alleles in HLA-A.

Table VIII represents the resolution that can be generated with the 19 markers for the distinction of the frequent HLA alleles in HLA-B.

Table IX represents the resolution that can be generated with the 10 markers for the distinction of the frequent HLA alleles in HLA-DRB1.

Table X represents the list of HLA-A alleles that are resolved with the 10 markers
for the creation of subgroups. Each subgroup is separated by an empty line.
Frequent alleles are shaded in darker grey, while lighter grey indicates the position that primers are extended onto.

Table XI represents the list of HLA-B alleles that are resolved with the 10 markers for the creation of subgroups. Each subgroup is separated by an empty line. Frequent alleles are shaded in darker grey, while lighter grey indicates the position that primers are extended onto.

Table XII represents the list of HLA-DRB1 alleles that are resolved with the 10 markers for the creation of subgroups. Each subgroup is separated by an empty line. Frequent alleles are shaded in darker grey, while lighter grey indicates the position that primers are extended onto.

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#### **Examples**

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Example 1: Mini-haplotyping at position 272 of HLA-B by the modified GOOD-Assay

A locus specific PCR product of exon 2 and exon 3 of HLA-B is amplified with a set of primers published by the International Histocompatibility Working Group, Technical Manuals (Hurly, Fernandes-Vina, Gao, Middleton, Noreen, Ren and Smith; www.ihwg.org/tmanual/Tmcontents.htm). The PCR product is incubated with SAP to remove all excess dNTPs. Then a single base primer extension at position 272 in the PCR amplicon is carried out. The set of primers, to generate the mini-haplotypes is shown in Table V. Thereafter a 5'phosphodiesterase digest is applied to reduce the primers to a core sequence. After alkylation of the DNA backbone of the mini-haplotype fragments the products are transferred onto a MALDI target pre-coated with matrix. Alternatively the matrix solution can be mixed with the samples and transferred onto the MALDI target to dry. The MALDI target is introduced into a MALDI mass spectrometer and analysed. The mass spectra show one or two mass peaks and that correspond to specific mini-haplotypes.

#### PCR:

Forward primer, BAmp1 5'-G GGT CCC AGT TCT AAA GTC CCC ACG-3'(1.875 pmol), reverse primer, BAmp2 5'-CC ATC CCC GGC GAC CTA TAG GAG ATG-3' (1.875 pmol) an BAmp3 5'-AGG CCA TCC CGG CGG GCG ATC TAT-3' (1.875 pmol), 0.25 μl 10x PCR buffer (HiFi Platinum Taq)), 0.3 μl MgSO<sub>4</sub> (50 mM), 0.2 μl of a mix of each dCTP, dATP, dGTP and dTTP (2 mM each), 0.25U engineered DNA polymerase (HiFi Platinum DNA Polymerase; Invitrogen) and 5 ng DNA fill to 3 μl with water. Cycling: 1. 94°C 3 min, 2. 94°C 20 sec, 3. 64°C 30 sec, 4. 72°C 30 sec, steps 2 to 4 are repeated 35 times, 5. 72°C 5 min.

#### SAP digest:

1.75  $\mu$ l of 50 mM Tris-HCl and 0.25  $\mu$ l SAP (USB corporation, Cleveland, USA) are to add to the PCR product and this has to be incubated for 60 min at 37°C, followed by an incubation at 90°C for 10 min to denature the SAP enzyme.

#### 5 Single Base Primer Extension:

To the SAP treated PCR product 2  $\mu$ l of an extension mix is to add. This mix contains 15 mM MgCl<sub>2</sub>, 0.1 mM of each of the four  $\alpha$ –S-ddNTPs, 5 pmol of the extension primers set and 0,4 U of Thermosequenase. Cycling: 1. 94°C 2 min, 2. 94°C 15 sec, 3. 58°C 20 sec, 4. 72°C 20 sec, steps 2 to 4 are repeated 50 times.

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### PDE digest:

To the extension product has to be added 0.5 ul 0.5 M acetic acid and 1.5  $\mu$ l PDE (5.1U) and incubate for at lease 120 min at 37 °C.

#### 15 Alkylation:

The alkylation is carried out by adding 21  $\mu$ l of an alkylation mix and incubate for 15 min at 40°C. Th alkylation mix contains 377 parts water free acetonitrile, 15 parts of 2M triethylamine/CO<sub>2</sub> (pH ~7.5), 75 parts 2mM Tris-HCl and 174 parts of methyliodine.

The alkylation is to stopped by adding 10  $\mu$ l deionised water. 5  $\mu$ l of the resulting upper phase are to dilute in 10  $\mu$ l 40% acetonitrile.

For MALDI target preparation and measurement with the MALDI mass spectrometer 0.5  $\mu$ l of the final dilution are transferred onto a MALDI target precoated with matrix ( $\alpha$ -cyano-4-hydroxycinnamic acid methyl ester). Measurement was carried out in a Bruker Autoflex with typically -18 kV acceleration voltage, pulsed ion extraction with a delay of 200 ns, and detection in linear detection mode. Results for CEPH family 1418 are shown in figure 6.

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Example 2: HLA-DR typing by the GOOD-Assay

A locus specific PCR for HLA-DRB is carried out. Therefore a set of allele-specific primers as listed below is used. These primers are published by J. Wu et al. in <a href="http://www.ihwg.org/tmanual/TMcontents.htm">http://www.ihwg.org/tmanual/TMcontents.htm</a> Chapter 10-B.

Name	Sequence
Amp1_DRB1_f20	5'-TTCTTGTGGSAGCTTAAGTT-3'
Amp2_DRB1_f21	5'-TTCCTGTGGCAGCCTAAGAGG-3'
Amp3_DRB1_f22	5'-CACGTTTCTTGGAGTACTCTAB-3'
Amp3-2_DRB1_f23	5'-CGTTTCTTGGAGTACTCTACGGG-3'
Amp3-3_DRB1_f23	5'-CGTTTCTTGGAGTACTCTACGTC-3'
Amp4_DRB1_f21	5'-GTTTCTTGGAGCAGGTTAAAC-3'
DR7_DRB1_£20	5'-CCTGTGGCAGGGTAARTATA-3'
DR9 DRB1 f18	5'-CCCAACCACGTTTCTTGA-3'
DR10 DRB1 fl9	5'-AGACCACGTTTCTTGGAGG-3'
AmpB DRB1_r18	5'-TCGCCGCTGCACYGTGAA-3'

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This set of primers carries a high risk of co-amplifying genes for the other HLA-DRB chains, which results in unclear results. However, this is currently the best available option for the PCR of HLA-DRB1. In order to resolve the problem an additional mini-haplotyping test can be added. The mini-haplotyping assay HLA-DRB\_122-126 gives good resolution of HLA-DRB genes and allows the verification of results produced for typing of HLA-DRB1 PCR products. The identification of HLA-DRB1 genes is possible, as well as the identification of other amplified HLA-DRB genes which are present is possible. The set of primers listed below is used for the primer extension reaction. The details of the protocol are identical to example 1.

			Masses				
Name	Sequence	CT	Primer	_A_	С	G	<u>T</u>
HLADR_1221_2f20	TGAAGAAATGACACTCAspTspG*spT	0	1487,5	- 1	-	_	1805,7
HLADR_1222_2f20	TGCAGAAATAGCACTCGspTspG*spT	o	1503,5	-	- 1	-	1821,7
HLADR_1223_2f20	TGAAGAAATGACACTCAspGspG*spT	0	1512,5		-	-	1830,7
HLADR_1224_2f20	TGAAGAAATGACACTTAspTspA*spT	0	1471,5	-	-	-	1789,7
HLADR_1225_2f20	TGAAGAAATGACACTCCspCspT*spC	-14	1510,6	- !	~	-	1814,8
HLADR_1226_2f20	TGAAGAAATRACACTCAspCspC*spC	-28	1418,4	1717,7	1693,6	1733,7	-
HLADR_1227_2f20	TGAAGAAATGACACTCAspTspA*spC	-14	1456,5	-	-	-	1760,7
HLADR_1228_2f20	TGAAGAAWTGACACTCAspGspA*spC	0	1481,5	-		-	1799,7
HLADR_1229_2f20	TGAGGAAATGACACTCAspCspA*spC	-14	1441,5	-	-	1770,8	1745,7
HLADR_12210_2f20	TGAAGATATGACACTCAspCspA*spC	-14	1441,5	-	-	1770,8	1745,7
HLADR 12211 2f20	TGAAGAAATGACAYTCAspAspA*spC	0	1465,5			<u> </u>	1783,7

Of the thirteen possible mini-haplotypes, four represent genes other than HLA-DRB1. The mini-haplotype GTGTT (1821.7 Da), AACAC in sense direction, represents with 100% certainty co-amplification of the HLA-DRB9 gene. The mini-haplotype ATACT (1760.8 Da), AGTAT in sense direction, represent either all HLA-DRB1\*07 alleles (except HLA-DRB1\*070102) or co-amplification of the HLA-DRB5 gene. The type TGTGT (1745.7 Da), AGTGT in sense direction, correspond to co-amplification or all variations of the HLA-DRB4 or HLA-DRB6 genes. Finally the type AGACT (1799.7 Da), AGTCT in sense direction, represent besides HLA-DRB1\*1130 and HLA-DRB1\*1446 also co-amplification of all variants of HLA-DRB3 and HLA-DRB7 genes.

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#### Claims

- 1. Method for HLA typing by the unambiguous determination of short DNA sequence elements (2-6 bases) at a given position simultaneously on both parental alleles at a selected number of positions in HLA genes, comprised of the steps for each position of a) hybridising a combination of oligonucleotides (primers) complementary to all known sequence variants to a DNA strand upstream of a given position; b) carrying out a primer extension reaction with at least one of the four dNTP substrates substituted by a terminating analog; c) analysing the products by mass spectrometry, with the resulting masses allowing unambiguous identification of the used primers and the added bases.
- 2. Method according to claim 1 where the DNA strand of step a) is produced by a DNA replication procedure such as PCR or rolling circle replication.
- 3. Method according to claim 1 where the combination of primers has slightly varying sequences so that all sequences of the haplotypes are represented by a perfectly matching primer.
  - 4. Method according to claim 3 where mass shifting tags are added to the individual primers sequences to make them uniquely distinguishable once the terminating base is added.
- 5. Method according to claim 1 where distinguishable termination products for known alleles are generated by extending the perfectly hybridised primer with a combination of dNTPs and ddNTPs or analogs thereof with a DNA polymerase to generate specific termination products.
  - 6. Method according to claim 1 where the GOOD assay is used.
- 7. Method according to any of the precedent claims where mass spectrometry, in particular MALDI or ESI mass spectrometry is used for analysis of the masses of products.
  - 8. Method for HLA typing according to any of the precedent claims above where set of multiple selected positions are queried to achieve sufficient information content.
  - 9. Method for HLA typing of HLA-A according to claims 1-8 where assays of the positions 98, 414, 539, 282, 571, 368, 256, 292, 238, 270, 453, 527, 502, 81,

- 268, 559, 92, 123 and 396 (according to the numbering of the HLA-A gene starting at cDNA sequence position 1 of exon 1) are used to achieve medium resolution.
- 10. Method for HLA typing of HLA-B according to claims 1-8 where assays of the positions: 539, 419, 559, 412, 272, 362, 302, 363, 206, 369, 259, 97, 583, 292, 222, 527, 418, 435 and 571 (according to the numbering of the HLA-B gene starting at cDNA sequence position 1 of exon 1) are used to achieve medium resolution.
- 11. Method for HLA typing of HLA-DRB1 according to claims 1-8 where assays of the positions 125, 196, 197, 227, 261, 286, 299, 308, 341 and 345 (according to the numbering of the HLA-DRB1 gene starting at cDNA sequence position 1 of exon 1) are used to achieve medium resolution.
  - 12. Method for HLA typing of HLA-A according to claims 1-8 where assays of the positions 98, 414, 539, 282, 571, 368, 256, 292, 238 and 270 (according to the numbering of the HLA-B gene starting at cDNA sequence position 1 of exon 1) are used to generate subgroups A-O.
- 13. Method for HLA typing according to claim 12 where assays of the positions 224, 268, 376, 502, 561 and 616 are preferably analysed to resolve subgroup HLA-A\_A; positions 126 and 526 to resolve subgroup HLA-A B; positions 81, 90, 92, 212, 214, 257, 265, 299, 302, 404, 420, 427, 453, 485, 489 and 502 to 20 resolve subgroup HLA-A\_C; positions 160, 200, 362 and 524 to resolve subgroup HLA-A\_D; positions 180, 299, 301, 302, 346, 418, 453, 517, 524, 526, 527, 557, 559 and 560 to resolve subgroup HLA-A E; positions 299, 301, 302, 341 and 583 to resolve subgroup HLA-A F; positions 127, 341, 399, 480, 502, 503, 524, 526, 527, 553, 559, 560 and 565 to resolve subgroup HLA-A G; 25 positions 228, 233, 463, 519, 530 and 583 to resolve subgroup HLA-A\_H; positions 102, 275, 317, 362, 418, 419, 497, 524, 555, 595 and 618 to resolve subgroup HLA-A\_I; positions 92, 331, 453, 524, 559, 560 and 564 to resolve subgroup HLA-A\_J; positions 78, 81, 123, 125, 142, 144, 194, 268, 294, 324, 355, 362, 396, 403, 419, 453, 456, 477, 493, 517, 524, 526, 527, 559 and 560 to 30 resolve subgroup HLA-A K; positions 113, 299, 301, 302, 308, 311, 523, 524 to resolve subgroup HLA-A\_L; positions 171, 363, 498 and 559 to resolve

- subgroup HLA-A\_M; positions 376, 426, 527, 555, 557 and 595 to resolve subgroup HLA-A\_N; position 299 to resolve subgroup HLA-A\_O are used.
- 14. Method for HLA typing of HLA-B according to claims 1-8 where assays of the positions 539, 419, 559, 412, 272, 362, 302, 363, 206 and 369 (according to the numbering of the HLA-B gene starting at DNA sequence position 1 of exon 1) are used to generate subgroups A-AC.
- 15. Method for HLA typing according to claim 14 where assays of the positions 259, 341 and 473 are preferably analyzed to resolve subgroup HLA-B\_A; positions 106, 144, 222, 259, 273, 311, 313, 418, 445, 493, 528 and 540 to resolve subgroup HLA-B B; positions 319, 416, 545 and 572 to resolve 10 subgroup HLA-B C; positions 106, 131, 165, 215, 243, 277, 292, 322, 481, 582, 603 and 616 to resolve subgroup HLA-B D; positions 106, 146, 165, 181, 238, 259, 263, 292, 328.1/329, 379, 435, 453, 463, 485, 526, 571, 572 and 583 to resolve subgroup HLA-B E; positions 142, 171, 255, 257, 395, 430, 544, 566 and 572 to resolve subgroup HLA-B\_F; positions 117, 247, 248, 277, 345, 418, 15 489 and 527 to resolve subgroup HLA-B G; positions 134, 141, 200, 213, 259, 304 and 527 to resolve subgroup HLA-B\_H; positions 83, 141, 211, 222, 242, 322, 404, 414, 435, 463, 502, 527, 544, 571, 572 and 583 to resolve subgroup HLA-B I; positions 103, 142, 222, 243, 259, 292, 477, 486 and 499 to resolve subgroup HLA-B J; positions 103, 259, 292, 295, 527 and 583 to resolve 20 subgroup HLA-B\_K; positions 320 and 500 to resolve subgroup HLA-B\_L; positions 311, 527 and 583 to resolve subgroup HLA-B\_M; positions 119, 292, 259, 319, 425, 527, 546 and 583 to resolve subgroup HLA-B\_N; positions 97, 142, 245 and 527 to resolve subgroup HLA-B O; positions 97 and 175 to resolve subgroup HLA-B P; positions 246 and 277 to resolve subgroup HLA-25 B Q; positions 246, 292, 311 and 503 to resolve subgroup HLA-B\_R; positions 103, 261, 309, 311 and 474 to resolve subgroup HLA-B\_S; positions 97, 103, 106, 243, 259, 292, 404 and 524 to resolve subgroup HLA-B\_T; positions 259 and 320 to resolve subgroup HLA-B\_U; position 106 to resolve HLA-B\_V; positions 97 to resolve HLA-B W; positions 97, 106, 257, 418 and 463 to 30 resolve HLA-B X; position 106 to resolve HLA-B Y; positions 106 and 144 to resolve HLA-B Z; positions 117, 247, 248, 283, 345, 418, 489, and 527 to

**\**\$\\$\

- resolve HLA-B\_AA; positions 106 to resolve HLA-B\_AB; positions 548 to resolve HLA-B\_AC.
- 16. Method of HLA typing according to claim 11 to resolve subgroups A-P of HLA-DRB1.
- 17. Method for HLA typing according to claim 16 where assays of the positions 5 123, 174, 250, 278 and 317 are analysed to resolve subgroup HLA-DRB1\_A; positions 192, 203, 256 and 259 to resolve subgroup HLA-DRB1 B; 256, 260, 317 and 351 to resolve subgroup HLA-DRB1 C; positions 155, 204, 233, 239, 256, 304, 357 and 366 to resolve subgroup HLA-DRB1\_D; positions 122, 171, 257 and 317 to resolve subgroup HLA-DRB1 E; positions 164, 167, 171, 230, 10 235, 306, 317, 321 and 337 to resolve subgroup HLA-DRB1 F; positions 164, 257, 266 and 303 to resolve subgroup HLA-DRB1\_G; positions 164, 181, 188, 220, 229, 256, 266, 317 and 318 to resolve subgroup HLA-DRB1 H; position 257 to resolve subgroup HLA-DRB1 I; positions 181, 239 and 357 to resolve subgroup HLA-DRB1 J; positions 122, 144, 239, 303, 317, 318 and 321 to 15 resolve subgroup HLA-DRB1 K; positions 118, 161, 257, 260, 318 and 321 to resolve subgroup HLA-DRB1 L; positions 165, 257, 293 and 303 to resolve subgroup HLA-DRB1 M; positions 177, 240, 256, 257 and 357 to resolve subgroup HLA-DRB1 N; positions 150 175, 230, 236 and 321 to resolve subgroup HLA-DRB1 O; positions 115, 220 and 317 to resolve subgroup HLA-20 DRB1 P are used.
  - 18. Kit for the implementation of the procedure according to claims 1 17 comprising pools of primers.
  - 19. Use of the method according to claims 1-17 for screening of tissue donors.
- 25 20. Use according to claim 19 for bone marrow donors in registries for screening of frequent and rare HLA types.
  - 21. Use of the primers represented in Table IV, V and VI to carry out HLA typing.

ATGGCCGTCATGGCGCCCCCGAACCCTCCTGCTACTCTCGGGGGCCCTGGCCCTGACCCAGACCTGGGCGGGTGAGTGCGGGGGTCGGGGAAACCGGCTCTGGGGGGGAAAC

\*123 ATCCGIGICCCGGCCCGGCGGGGGAGCCCCCGCTICAICGCGTGGGCIACGIGGACGACGCGGTICGIGCGGTICGACAGGGGCCGCGGGGCCAGAAGAIGGAGCCGCGG 

368\* GACCGCGGGGTCGGGGTTCTCACACCATCCAGATAA<mark>ITGAA</mark>TGGCTGCGACGTGGGCCGGACGGGCG<mark>CATG</mark>GTCGGGGTA<mark>CGG</mark>CAGGACGCCTACGACGGCAAGGAT

TGGCCTCCCACAAGGAGGGGGAGACAATTTGGGAACCACTAGAATATCACCCTCCTCTG

\*123 ATCCGIGICCCGGCCCG<mark>GGGGG</mark>AGCCCCCGCTTCATCGCGTGGGCTACGTGGACGACGCAGTTCGTGCGGTTCGACAGCGACGCGGGGGAGCCAGAAGATGGAGCCGGGG

\*396 GACCGC*GGGGTCGGGCCCAGGITCICACAICAICALAAITAI*IGGCIGCGACGIGGGGCCGGACGGGCG<mark>C</mark>IIGGICGGGTA<mark>GGG</mark>CAGGACGCCIACGACGGCAAGGAI

\*453 \*453 TACATCGCCCTGAA<mark>GGA</mark>GACCTGCGCTCTTGGACCGCGGGGGACATGGCAGATCACC<mark>AAGGCG</mark>CAAGTGGGAGGCGGTCC<mark>ATGC</mark>GGCGAGGAGGAGGTCTACCTGG

\*559 AGGGG<mark>GGT</mark>GCGT<u>GGAGG</u>GGCTCCGCAGATACCTGGAAAGGAAGGAAGGAGGCGCTGCAGGGTACCAGGGGGCGCCACGGGGCCCCTCCTGATCGCTTAGATCTCCGGGGC

TGGCCTCCCACAAGGAGGGGAGACAATTTGGGGAGCAACACTAGAATATCACCCTCCTCTG

# IGURE 2

## FIGURE 3

CTTCATCTCAGGGCTACGIGGACGACACCCAGTTCGIGAGGTTCGACAGCGACGCCGCGAGTCCGAGGGGGGGCGCGCGGCGCGCGTCGATAGAGCAGGGGGGCGGGAGTAT

CGCAGGTCACGACTCCCCATCCCCCACGTACGGCCCCGGGTCGCCCCGAGTCTCGGGGTCCGGCTCCCTCAGGGCCGGGGGACCCGCCCAGACCTTCGACCGGCGAGACC

362\*\*363 \*369 ACCCTC<u>CAGA**GC**ATG</u>TA<mark>GGGC</mark>TGCGACGGGGCGCCCCTCCTCCTCCGGGGAAGGAAGGCCGTACGACGCAA<mark>GGAT</mark>TACATCGCCCTGAAGGACCTGCGCT

.

## FIGURE

362\*\*363 \*369 ACCĆTC<u>CAGAGCATG</u>TA<mark>GGG</mark>TGCGACGGGCGCCGCGCCTCCTCCGCGGGGATG</mark>ACCAGT<mark>AGGCC</mark>TACGACGCAAGGATTACATCGCCCTGAACGAGCACTGCGCT

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196\*\*197

IGURE 5

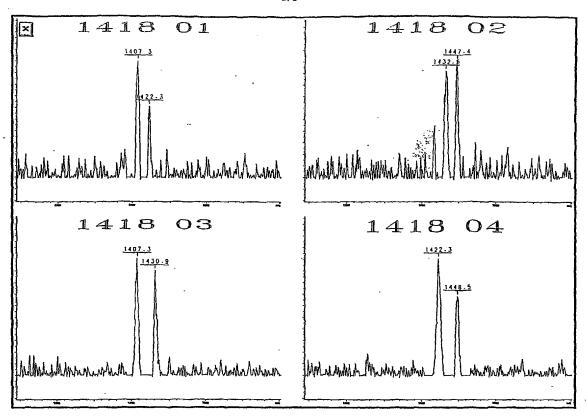


FIGURE 6

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BNSDOCID: <WO\_\_\_\_2005052189A2\_I\_>

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	WO 2005/052189		PCT/IB2004/004115
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BNSDOCID: <WO\_\_\_\_2005052189A2\_1\_>

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# (19) World Intellectual Property Organization

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- (71) Applicant (for all designated States except US): CONSORTIUM NATIONAL DE RECHERCHE EN GENOMIQUE (CNRG) [FR/FR]; 2, rue Gaston Crémieux, F-91000 Evry (FR).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): GUT, Ivo, Glynne [GB/FR]; 18, rue du Moulin Vert, F-75014 Paris (FR). KUCHARZAK, Ramon [FR/FR]; 56, rue Olivier Metra, F-75020 Paris (FR).
- (74) Agents: MARTIN, Jean-Jacques et al.; Cabinet Regimbeau, 20, rue de Chazelles, F-75847 Paris Cedex 17 (FR).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

## Published:

with international search report

(88) Date of publication of the international search report: 20 October 2005

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHOD FOR HLA TYPING

(57) Abstract: A method for the identification of DNA sequence elements in complex and highly variable sequences is described. The method consists of identifying a short sequence element of several DNA bases (2-6 bases) at a given position in the genome simultaneously on all parental alleles. The method allows differentiating mini-haplotypes on different alleles in one analysis. The method consists of carrying out an enzymatic primer extension reaction with a combination of extension primers (pool of primers) and analysing the products by mass spectrometry. The pool of primers is assembled in such a way that the primer extension product allows unambiguous identification of both the primer of the pool that was extended and the base that was added. The method is of great utility for DNA sequences harbouring many SNPs close to each other with many possible haplotypes. Such sequences are known in the Major Histocompatibility Complex (MHC). This method is particularly well suited for DNA-based HLA typing and in combination with a suitable selection of sites tested, it is superior in ease of operation to conventional HLA typing methods. We have identified sets of these assays for HLA-A, HLA-B, and HLA-DRB 1 that allow unambiguous four-digit HLA of each of these genes with between 11 and 28 queried markers.

Internation Application No PCT/IB2004/004115

A. CLASSIFICATION OF SUBJECT MATTER IPC 7 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  $IPC \ 7 \ C12Q$ 

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, EMBASE, CHEM ABS Data, EMBL

C. DOCUM	ENTS CONSIDERED TO BE RELEVANT	
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Χ	PASTINEN T ET AL: "Multiplex,	18
	fluorescent, solid-phase minisequencing	
	for efficient screening of DNA sequence variation"	
	CLINICAL CHEMISTRY, AMERICAN ASSOCIATION	-
	FOR CLINICAL CHEMISTRY. WINSTON, US,	
	vol. 42, no. 9, 1996, pages 1391-1397,	
	XP002126144	
	ISSN: 0009-9147	1 0 10
Y	page 1392, left-hand column; table 1	1-8,19, 20
X	WO 00/65088 A (AMERSHAM PHARM BIOTECH AB ;	18
	ULFENDAHL PER JOHAN (SE); WONG KIN CHUN (S) 2 November 2000 (2000-11-02)	
	claims 12,14,21	1 0 10
Y	the whole document	1-8,19, 20
	-/	

X Further documents are listed in the continuation of box C.	Patent family members are listed in annex.
<ul> <li>Special categories of cited documents:</li> <li>'A" document defining the general state of the art which is not considered to be of particular relevance</li> <li>'E" earlier document but published on or after the international filling date</li> <li>'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</li> <li>'O' document referring to an oral disclosure, use, exhibition or other means</li> <li>'P' document published prior to the international filing date but later than the priority date claimed</li> </ul>	<ul> <li>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</li> <li>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</li> <li>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</li> <li>"&amp;" document member of the same patent family</li> </ul>
Date of the actual completion of the international search	Date of mailing of the international search report  1 1. 07. 2005
11 March 2005  Name and mailing address of the ISA  European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Authorized officer  Hagenmaier, S

Form PCT/ISA/210 (second sheet) (January 2004)

Internal al Application No
PCT/IB2004/004115

C.(Continu	ation) DOCUMENTS CONSIDERED TO BE RELEVANT	/182004/004115
Category °		Relevant to claim No.
Υ	WORPALL T A ET AL. "Allele-specific	1_0
ı	WORRALL T A ET AL: "Allele-specific HLA-DR typing by mass spectrometry: an alternative to hybridization-based typing methods."	1-8, 18-20
	ANALYTICAL CHEMISTRY. 1 NOV 2000, vol. 72, no. 21,	
	1 November 2000 (2000-11-01), pages 5233-5238, XP002287583 ISSN: 0003-2700	
Α	the whole document	9,12,13
Y	LEUSHNER JAMES ET AL: "Automated mass spectroscopic platform for high throughput DR Beta typing" HUMAN IMMUNOLOGY, vol. 61, no. Supplement 2, 2000, page S126, XP008032510	1-8, 18-20
	& 26TH ANNUAL MEETING OF THE AMERICAN SOCIETY FOR HISTOCOMPATIBILITY AND IMMUNOGENETICS; LAKE BUENA VISTA, FLORIDA, USA; OCTOBER 10-14, 2000	
Α	ISSN: 0198-8859 abstract	9,12,13
Y	TOST J ET AL: "GENOTYPING SINGLE NUCLEOTIDE POLYMORPHISMS BY MASS SPECTROMETRY" MASS SPECTROMETRY REVIEWS, JOHN WILEY AND SONS, NEW YORK, NY, US,	1-8, 18-20
A	vol. 21, no. 6, November 2002 (2002-11), pages 388-418, XP009019382 ISSN: 0022-7037 the whole document	9,12,13
Υ	TOST JÖRG ET AL: "Molecular haplotyping	1-8,
	at high throughput." NUCLEIC ACIDS RESEARCH. 1 OCT 2002, vol. 30, no. 19,	18–20
	1 October 2002 (2002-10-01), page e96, XP002287584 ISSN: 1362-4962	
Α	the whole document	9,12,13
Υ	SAUER S ET AL: "EXTENSION OF THE GOOD ASSAY FOR GENOTYPING SINGLE NUCLEOTIDE POLYMORPHISMS BY MATRIX-ASSISTED LASER DESORPTION/IONIZATION MASS SPECTROMETRY" RAPID COMMUNICATIONS IN MASS SPECTROMETRY, HEYDEN, LONDON, GB, vol. 17, no. 12, 9 May 2003 (2003-05-09),	1-8, 18-20
Α	pages 1265-1272, XP009019406 ISSN: 0951-4198 the whole document	9,12,13
п	-/	9,12,13
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Internal Application No
PCT/IB2004/004115

1		PC1/1B2004/004115
<del> </del>	ation) DOCUMENTS CONSIDERED TO BE RELEVANT	
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SAUER SASCHA ET AL: "Genotyping single-nucleotide polymorphisms by matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry."  JOURNAL OF CHROMATOGRAPHY. B, ANALYTICAL TECHNOLOGIES IN THE BIOMEDICAL AND LIFE SCIENCES. 25 DEC 2002, vol. 782, no. 1-2, 25 December 2002 (2002-12-25), pages 73-87, XP002287585	1-8, 18-20
Α	ISSN: 1570-0232 the whole document	9,12,13
Y	WO 02/08462 A (LECHNER DORIS; GUT IVO GLYNNE (FR); CT NAT DE GENOTYPAGE (FR)) 31 January 2002 (2002-01-31)	1-8, 18-20
<b>A</b> .	the whole document	9,12,13
Y	ROZEMULLER: "Reference panels for sequence based typing: Selection criteria for HLA-A and HLA-B" 2000, , XP002287586 ISBN: 0-945278-02-0 Retrieved from the Internet: URL:http://www.ihwg.org/tmanual/TMcontents .htm> 'retrieved on 2004-07-05!	1-8, 18-20
A	Chapter 1-B	9,12,13
Y A	WO 02/18659 A (HAPLOGEN LLC ; LIU XIANGJUN (US)) 7 March 2002 (2002-03-07) the whole document	1-8, 18-20 9,12,13
Y A	US 5 451 512 A (APPLE RAYMOND J ET AL) 19 September 1995 (1995-09-19) the whole document	1-8, 18-20 9,12,13
	4:	

Form PCT/ISA/210 (continuation of second sheet) (January 2004)

International application No.

PCT/IB2004/004115

Вох	No. I	Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)
1.	With inven	regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed tion, the international search was carried out on the basis of:
	a.	type of material  X a sequence listing table(s) related to the sequence listing
	b.	format of material    X   in written format     X   in computer readable form
2.	c.	time of filing/furnishing  X contained in the international application as filed  X filed together with the international application in computer readable form  furnished subsequently to this Authority for the purpose of search  In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed
	_	or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3.	Addit	ional comments:

Form PCT/ISA/210 (continuation of first sheet (1)) (January 2004)

International application No. PCT/IB2004/004115

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)
This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:
Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
Claims Nos.:     because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).
Box III Observations where unity of invention is lacking (Continuation of Item 3 of first sheet)
This International Searching Authority found multiple inventions in this international application, as follows:
see additional sheet
As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the Invention first mentioned in the claims; it is covered by claims Nos.:  Claims 1-8, 18-20 (all partially), 9, 12, 13 (completely)
Remark on Protest  The additional search fees were accompanied by the applicant's protest.  No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (2)) (January 2004)

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

donors.

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

Invention 1: claims 1-8, 18-20 (all partially), 9,12,13 (completely)

Method for HLA typing of HLA-A by the unambiguous determination of short DNA sequence elements at positions 98, 414,539,282,571,368,256,292,238 and 270 simultaneously on both parental alleles at a selected number of positions in HLA -A, comprised of the steps for each position a) hybridising a combination of oligonucleotides complementary to all known sequence variants to a DNA strand upstream of a given position b) carrying out a primer extension reaction with at least one of the four dNTP substrates substituted by a terminating analog c) analysing the products by mass spectrometry, with the resulting masses allowing unambiguous identification of the used primers and added bases; kit for the implementation of such method; use of such method for screening of tissue

Invention 2: 1-8, 18-20 (all partially), 10,14,15 (completely)

Method for HLA typing of HLA-B by the unambiguous determination of short DNA sequence elements at positions 539,419,559,412,272,362,302,363,206 and 369 simultaneously on both parental alleles at a selected number of positions in HLA-B, comprised of the steps for each position a) hybridising a combination of oligonucleotides complementary to all known sequence variants to a DNA strand upstream of a given position

b) carrying out a primer extension reaction with at least one of the four dNTP substrates substituted by a terminating analog

c) analysing the products by mass spectrometry, with the resulting masses allowing unambiguous identification of the used primers and added bases; kit for the implementation of such method; use of such method for screening of tissue donors.

Invention 3: claims 1-8, 18-20 (all partially), 11,16,17 (completely)

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Method for HLA typing of HLA-DRB1 by the unambiguous determination of short DNA sequence elements at positions 125,196,197,227,261,286,299,308,341 and 345 simultaneously on both parental alleles at a selected number of positions in HLA-DRB1, comprised of the steps for each position a) hybridising a combination of oligonucleotides complementary to all known sequence variants to a DNA strand upstream of a given position

- b) carrying out a primer extension reaction with at least one of the four dNTP substrates substituted by a terminating analog
- c) analysing the products by mass spectrometry, with the resulting masses allowing unambiguous identification of the used primers and added bases; kit for the implementation of such method; use of such method for screening of tissue donors.

Inventions 4-246: claim 21 (partially)

Invention 4:

Use of the primer with Seq.ID 1 to carry out HLA typing. ..ibidem for inventions 5-246, i.e. each of the 242 primers listed in table IV,V and VI.

Information on patent family members

Internation No
PCT/IB2004/004115

Patent document cited in search report		Publication date	_	Patent family member(s)	Publication date
WO 0065088	Α	02-11-2000	AU WO	5062500 A 0065088 A2	10-11-2000 02-11-2000
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Form PCT/ISA/210 (patent family annex) (January 2004)

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<sup>3</sup>			